

Science

4 October 2013 | \$10



Communication in Science

Pressures
and
Predators

 AAAS

SPECIAL SECTION

Communication in Science

INTRODUCTION

- 56 Scientific Discourse: Buckling at the Seams
R. Stone and B. Jasny

NEWS

- 58 The Rise of Open Access
56 Who's Afraid of Peer Review?
 >> *Science Podcast*
66 The Seer of Science Publishing
68 The Power of Negative Thinking
 >> *Science Podcast*
70 Hey, You've Got to Hide Your Work Away

- 72 Lock Up the Genome, Lock Down Research?

- 74 The Annual Meeting:
Improving What Isn't Broken
 >> *Science Podcast*

POLICY FORUM

- 80 Scholarly Communication:
Cultural Contexts, Evolving Models
D. Harley
 >> *Editorial p. 13; Perspectives pp. 44, 49, and 53;*
 Report p. 127; Science Careers, Survey, Interactive,
 and Podcast at www.sciencemag.org/special/scicomm

EDITORIAL

- 13 Improving Scientific Communication
Marcia McNutt
 >> *Communication in Science section p. 56*

NEWS OF THE WEEK

- 20 A roundup of the week's top stories

NEWS & ANALYSIS

- 22 U.S. Shutdown Spares an 'Essential' Few
23 The IPCC Gains Confidence in Key Forecast
 A Stronger IPCC Report
24 For Researchers, IPCC Leaves
 a Deep Impression
25 Brain Stimulation Sparks
 'Machiavellian' Choices Over Money
26 Large-Scale Gene Comparisons Promise
 to Boost Tree of Life Studies and More
27 NIH Seeks Better Database
 for Genetic Diagnosis

NEWS FOCUS

- 29 The Art of Eradicating Polio

LETTERS

- 36 NextGenVoices

BOOKS ET AL.

- 39 From Strange Simplicity
to Complex Familiarity
M. Eigen, reviewed by A. Traulsen
40 The Power Surge
M. Levi, reviewed by S. H. Ali

POLICY FORUM

- 41 Managing Forests and Fire
in Changing Climates
S. L. Stephens et al.

PERSPECTIVES

- 44 Future Science
J. A. Evans
 >> *Communication in Science section p. 56;*
 Report p. 127
45 Small RNA—the Secret of Noble Rot
D. Baulcombe
 >> *Report p. 118*
46 Turn the Molecule This Way
for a Faster Reaction
M. C. Heaven
 >> *Report p. 98*
47 Social Factors in Epidemiology
C. T. Bauch and A. P. Galvani
 >> *Perspective p. 53*

- 49 Public Science 2.0—Back to the Future
C. Könniker and B. Luger
 >> *Communication in Science section p. 56*

- 50 Fine-Tuning Photosynthesis
J.-D. Rochaix
 >> *Report p. 114*

- 52 A Looser Clock to Cure Jet Lag
M. H. Hastings
 >> *Research Article p. 85*

- 53 A Risky Science Communication
Environment for Vaccines
D. M. Kahan
 >> *Perspective p. 47; Communication in Science*
 section p. 56; Science Podcast

CONTENTS continued >>

ON THE WEB THIS WEEK

>> Science Podcast

Listen to a special show dedicated to science communication in which we explore pressures and predators.

>> Find More Online

Check out *Science Express*, our podcast, videos, daily news, our research journals, and *Science Careers* at www.sciencemag.org.

DEPARTMENTS

- 11 This Week in *Science*
15 Editors' Choice
18 *Science* Staff
133 New Products
134 *Science Careers*



COVER

The ability to publish papers and underlying data in full on the Internet is changing how scientists communicate. However, trust in the integrity of submissions and in peer reviewers is being tested by a recent disruptive change: open access. In a special section, *Science* probes the dramatic shifts in the landscape of scientific communication. See page 56.

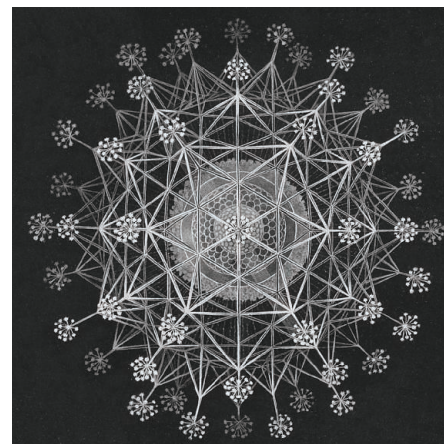
Image: David Plunkert

RESEARCH ARTICLES

- 84** Integrative Annotation of Variants from 1092 Humans: Application to Cancer Genomics
E. Khurana et al.
Regions under strong selection in the human genome identify noncoding regulatory elements with possible roles in disease.
Research Article Summary; for full text: <http://dx.doi.org/10.1126/science.1235587>
- 85** Mice Genetically Deficient in Vasopressin V1a and V1b Receptors Are Resistant to Jet Lag
Y. Yamaguchi et al.
In mice, the pace of recovery from jet lag is partly determined by vasopressin signaling in a certain region of the brain.
>> *Perspective p. 52*

REPORTS

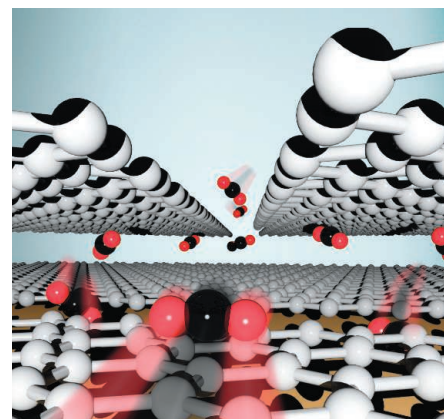
- 91** Selective Gas Transport Through Few-Layered Graphene and Graphene Oxide Membranes
H. W. Kim et al.
Stacked graphene and graphene oxide membranes prepared with gas flow channels exhibit tunable gas separation performance.
- 95** Ultrathin, Molecular-Sieving Graphene Oxide Membranes for Selective Hydrogen Separation
H. Li et al.
Ultrathin graphene oxide membranes show enhanced separation selectivity for hydrogen gas.
- 98** Specific Chemical Reactivities of Spatially Separated 3-Aminophenol Conformers with Cold Ca^+ Ions
Y.-P. Chang et al.
A molecular beam technique measures the different reactivities of a compound's distinct rotational conformations.
>> *Perspective p. 46*
- 101** Nitrogen Isotopic Composition and Density of the Archean Atmosphere
B. Marty et al.
Earth's Archean atmosphere contained roughly as much nitrogen between 3.0 and 3.5 billion years ago as it does today.
- 104** Following Gene Duplication, Paralog Interference Constrains Transcriptional Circuit Evolution
C. R. Baker et al.
Interactions between recent gene duplicates may create functional interference, selecting for regulatory complexity.
- 108** Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs
J. M. de Goeij et al.
Sponges take up dissolved organic matter and convert it into consumable cellular material.
- 111** Allele-Specific Silencing of Mutant *Myh6* Transcripts in Mice Suppresses Hypertrophic Cardiomyopathy
J. Jiang et al.
In a mouse model, heart disease can be delayed by a therapy that prevents expression of the disease-causing mutation.
- 114** A Thylakoid-Located Two-Pore K^+ Channel Controls Photosynthetic Light Utilization in Plants
L. Carraretto et al.
The electrochemical gradient used to make adenosine triphosphate in photosynthesis is modulated by potassium counterflow.
>> *Perspective p. 50*
- 118** Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways
A. Weiberg et al.
A pathogenic fungus delivers small RNA molecules to disable gene regulatory systems in the target plant.
>> *Perspective p. 45*
- 123** Crystal Structure of Na^+ , K^+ -ATPase in the Na^+ -Bound State
M. Nyblom et al.
The location of three bound sodium ions and the mechanism of sodium release in a key plasma membrane ion pump are revealed.
- 127** Quantifying Long-Term Scientific Impact
D. Wang et al.
Early citation history can be used to model the total number of citations a paper will receive and to compare journals.
>> *Perspective p. 44; Communication in Science section p. 56*



page 39



pages 52 & 85



pages 91 & 95

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2013 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$149 (\$74 allocated to subscription). Domestic institutional subscription (51 issues): \$990; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. Postmaster: Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. Single-copy sales: \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$30.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.

<< Sponge Pump

"Darwin's Paradox" asks how productive and diverse ecosystems like coral reefs thrive in the marine equivalent of a desert. **De Goeij et al.** (p. 108) now show that coral reef sponges are part of a highly efficient recycling pathway for dissolved organic matter (DOM), converting it, via rapid sponge-cell turnover, into cellular detritus that becomes food for reef consumers. DOM transfer through the sponge loop approaches the gross primary production rates required for the entire coral reef ecosystem.

Gas Separations

When gas separation membranes are made thinner, they usually allow permeating gases to pass through faster. However, a thinner membrane may be poorer at separating between gas species. **Kim et al.** (p. 91) examined the permeability and selectivity of layered graphene and graphene oxide membranes. Gas molecules diffuse through defective pores and channels that form between the layers. Controlling these structures tuned the properties of the membranes to allow the extraction of carbon dioxide from other gases. **Li et al.** (p. 95) describe membranes as thin as 1.8 nanometers made from only two to three layers of graphene oxide. Small defects within the layers allowed hydrogen to pass through, separating it from carbon dioxide and nitrogen.

Resetting the Circadian Clock

Fatigue and other symptoms of jet lag arise when the body's internal circadian clock is out of sync with environmental light-dark cycles. Studying genetically modified mice lacking two receptors for the peptide hormone vasopressin under experimental conditions simulating jet lag, **Yamaguchi et al.** (p. 85; see the Perspective by **Hastings**) concluded that vasopressin signaling in the suprachiasmatic nucleus (SCN)—a region of the brain known to control circadian rhythms—impedes adjustment to the environmental clock. Infusion of vasopressin receptor antagonists directly into the SCN of wild-type mice accelerated their recovery from

jet lag, suggesting that this pathway may merit further investigation as a pharmacological target for treating jet lag.

Same As It Ever Was

Nitrogen constitutes approximately 78% by volume of Earth's atmosphere and is a key component in its chemical and physical characteristics. It is not clear whether N_2 has been so abundant throughout Earth's geological history. **Marty et al.** (p. 101, published online 19 September) analyzed the isotopic compositions of nitrogen and argon from fluid inclusions trapped in hydrothermal quartz formed 3 to 3.5 billion years ago. The partial pressure and isotopic composition of atmospheric N_2 were similar to today's. Thus, other factors are needed to explain why liquid water existed on Earth's surface despite the Sun being 30% less luminous.

Silencing a Silent Killer

Hypertrophic cardiomyopathy (HCM) is a leading cause of sudden death in young athletes. HCM is caused by dominant mutations in genes encoding constituents of the cardiac sarcomere, the contractile unit that keeps the heart pumping. Studying a mouse model that recapitulates a severe form of HCM caused by a mutation in a β myosin heavy chain gene, **Jiang et al.** (p. 111) investigated whether sarcomere dysfunction could be corrected by selectively silencing expression of the mutant allele. Mice treated shortly after birth with a viral vector encoding an appropriately designed RNA interference cassette

did not develop cardiac hypertrophy or myocardial fibrosis—the pathologic manifestations of HCM—for at least 6 months.

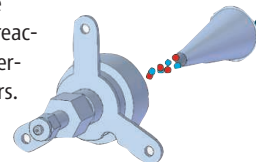
RNA on the Attack

Plant microbial pathogens often work through protein effectors that are delivered into the plant cells to disrupt critical cellular functions. **Weiberg et al.** (p. 118; see the Perspective by **Baulcombe**) have now found that small RNAs (sRNAs) of the fungus *Botrytis cinerea* can play a similar role. After fungal infection of tomato or *Arabidopsis* leaves, the plant cells contained a suite of fungal-derived sRNAs. Three sRNAs were found to bind to the plant's own Argonaute protein, thereby silencing the plant's fungal defense genes.

Reactive Conformations

Most molecules manifest a fair amount of flexibility at room temperature, in particular through interconversion of rotational conformers—structures that differ by the relative orientation of groups on either side of a single covalent bond. **Chang et al.** (p. 98; see the Perspective by **Heaven**) devised a method to explore the comparative reactivities of different conformers.

A mixture of the conformers was prepared in a molecular beam cold enough to preclude interconversion; then an electric field was used to push the different conformers apart, spatially resolving subsequent collisional interactions with a target of trapped ions.



Citation Grabbers

Is there quantifiable regularity and predictability in citation patterns? It is clear that papers that have been cited frequently tend to accumulate more citations. It is also clear that, with time, even the most novel paper loses its currency. Some papers, however, seem to have an inherent "fitness" that can be interpreted as a community's response to the research. **Wang et al.** (p. 127; see the Perspective by **Evans**) developed a mechanistic model to predict citation history. The model links a paper's ultimate impact, represented by the total number of citations the paper will ever receive, to a single measurable parameter inferred from its early citation history. The model was used to identify factors that influence a journal's impact factor.

Additional summaries

Identifying Important Identifiers

Each of us has millions of sequence variations in our genomes. Signatures of purifying or negative selection should help identify which of those variations is functionally important. **Khurana *et al.*** (p. 84) used sequence polymorphisms from 1092 humans across 14 populations to identify patterns of selection, especially in noncoding regulatory regions. Noncoding regions under very strong negative selection included binding sites of some chromatin and general transcription factors (TFs) and core motifs of some important TF families. Positive selection in TF binding sites tended to occur in network hub promoters. Many recurrent somatic cancer variants occurred in noncoding regulatory regions and thus might indicate mutations that drive cancer.

Two Are Not Necessarily Better Than One

Gene duplication is one of the major drivers of the evolution of gene and protein networks. However, specific examples of how genes change to maintain two paralogous gene copies within an organism are relatively rare. **Baker *et al.*** (p. 104) examined the functional divergence of the paralogs Mcm1 and Arg80, MADS-box

transcription factors in the yeast *Saccharomyces cerevisiae*. The partitioning of ancestral functions in a fungal transcription factor appeared to be affected by competitive interference between the newly formed gene duplicates. Thus, in yeast, gene duplication has created a selective conflict between the paralogs, which appear to have driven the evolution of a novel protein function.

pH Gradient in Light of Electroneutrality

Photosynthesis in plant chloroplasts depends on a proton gradient to convert light energy into adenosine triphosphate. Studying *Arabidopsis*, **Carraretto *et al.*** (p. 114, published online 5 September; see the Perspective by **Rochaix**) identified the potassium channel TPK3 in the stacked membranes of the chloroplast's thylakoids as key to sustaining the proton gradient. As the thylakoid lumen acidifies on exposure to light, electroneutrality derives from TPK3 activity. TPK3 was

able to optimize chloroplast responses to light across a wide range of intensities. Plants lacking functional TPK3 appeared normal when grown at modest light levels, but at higher light levels, the plants showed disruptions in overall growth and in thylakoid organization.

Pumping Out Sodium

Mammalian cells contain relatively high concentrations of potassium but low concentrations of sodium. This balance is maintained by an ion pump, the Na⁺, K⁺-adenosine triphosphatase, in an adenosine triphosphate-driven transport cycle that results in the export of three sodium ions and the import of two potassium ions. Structures of potassium-bound conformations of the pump have been determined. Now, **Nyblom *et al.*** (p. 123, published online 19 September) report on the high-resolution crystal structure of a Na⁺-bound conformation, which reveals conformational changes associated with Na⁺ binding.





Marcia McNutt is Editor-in-Chief of *Science*.

Improving Scientific Communication

EVEN THE MOST BRILLIANT SCIENTIFIC DISCOVERY, IF NOT COMMUNICATED WIDELY AND ACCURATELY, is of little value. And with the explosion of science around the globe, the dissemination of scientific information, once the purview of learned societies and a handful of publishers, is now a growth industry. This growth has attracted new models and new providers of services. In the process, the standards for scientific communication are slipping (see the special section on Communication in Science beginning on p. 56). The science community must explore new ways to improve upon them.

Why are standards important? For science professionals, time is a very precious commodity. The peer-review process provides some level of assurance about the accuracy of a study's conclusions, relieving each scientist from having to assess the veracity of each paper personally. Through the efforts of independent experts and editors, peer review raises the quality of what is ultimately published. For nonscientists, peer-reviewed publications are considered the "gold standard" of trusted material for policy, management, and other applications of science.

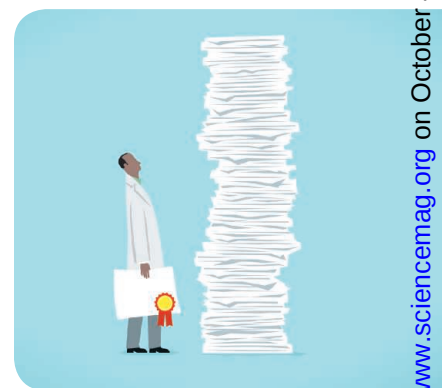
Yet, for a profession that prides itself on the application of experimentation and observation to find truth, scant attention has been paid to improving the institution of peer review, despite the pressure to evolve this time-honored tradition. Much of the growth in journals has been in open-access titles, a trend that has improved access to scientific information. But the open-access business model depends on a high volume of published papers for financial viability, leaving little time for the deliberative process of traditional peer review. Some open-access journals that promise peer review fail to deliver it (see the News story by Bohannon on p. 60).

Novel ways to streamline the review process have been proposed, such as having authors solicit and pay for their own reviews. For the most part, the new schemes lack the sort of "double-blind" tests that scientists would expect, in a drug trial, for example. In such a test, both the author revising the paper and the judges determining which papers are most improved through the review process are blind to which method of peer review is applied. I propose that the science community explore more of these alternatives, but also consider how the effectiveness of new reviewing methods can be rigorously assessed. Which maintain or improve quality standards? Are some better suited to various open-access models of publishing?

Even before scientific material is published, the first outlet for communication is typically a scientific meeting. All presenters want to describe their findings to an audience of influential luminaries in their fields, and they certainly would be disappointed if a conference billed as a gathering of experts were nothing of the sort (see the News story by Cohen on p. 76). Travel budgets are so meager that scientists must carefully prioritize what meetings they attend. On the other hand, much of the growth in science overall has been in nations that, until recently, rarely hosted international meetings. It is understandable that organizations within those countries would want to attract outside scientists to present papers, to benefit their own national efforts. Again, there is scant evidence on how to best use scientific meetings to build an international community. What meeting sizes work the best? What is the best mix of students and established researchers? What assures someone of the quality of a meeting before they commit to attend (such as a peer-reviewed program)? What venues are best for particular types of meetings? Is it better to limit the number of concurrent sessions at the expense of a longer meeting? How does the experience of remote attendees (viewing sessions online) compare to that of in-person attendees? How can that experience at a distance be improved?

It is high time that scientists apply scientific thinking to determine how to better communicate their science. Science progresses through experimentation and evidence. I would like to think that science communication can as well.

— Marcia McNutt



MATERIALS SCIENCE

3D Lowers *T*

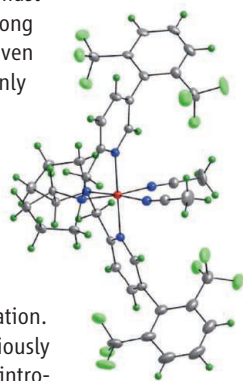
Most solid oxide fuel cells operate at temperatures above 800°C, necessitating robust supporting materials for insulation and rendering on/off cycling inefficient. Lower-temperature operation has tended to lead to a loss of catalytic activity. An *et al.* combined several approaches to create a cell that performs well at temperatures as low as 450°C. At the core is a three-dimensional (3D) membrane electrode assembly (MEA) structure, which increases the surface area for the catalytic reactions. On both the top and bottom surfaces, a layer of porous platinum was deposited, further enhancing the reaction area. The addition of yttria-doped ceria at the cathode interface of the MEA below the top platinum surface helped reduce activation losses by 35%. The open circuit voltage was measured to be close to the thermodynamic limit, showing electronic and chemical isolation of the cathode and anode. The work is still at an early stage, though, as the performance of the MEA dropped by about 30% during the first hour of operation, possibly due to changes in structure, but did not show further changes after another 2 hours of operation. — MSL

Nano Lett. **13**, 4551 (2013).

CHEMISTRY

Telling O Where to Go

The abundance of aliphatic C-H bonds in organic molecules poses an enticing, yet maddening challenge to synthetic chemists. On the one hand, direct oxidation protocols are prospectively applicable to an immense range of substrates; on the other hand, genuinely useful methods must achieve selectivity among numerous sites in a given substrate that differ only subtly. Gormisky and White tackle this challenge through the use of a pair of complementary ligands on an iron catalyst that activates peroxide for C-H oxidation. Elaborating on a previously reported ligand, they introduce bulky bis(trifluoromethyl) phenyl groups that roughly halve the conical angle-bounding substrate approach to the metal center. As a result, this bulkier catalyst favors oxidation at sterically unhindered sites on a substrate, whereas the previous catalyst manifested selectivity governed by



the substrate's inherent reactivity. The authors develop a predictive model for the site preferences of each ligand based on steric and electronic factors, and they validate it through site-selective oxidations of such complex molecules as artemisinin (where they can select between two of nine possible sites) and the pentacyclic triacetoxyltricalysiolide. — JSY

J. Am. Chem. Soc. **135**, 10.1021/ja407388y (2013).

VIROLOGY

Experimental Intervention

We know that some viruses can rapidly spread among wild birds, as witnessed by pandemic influenza viruses. Realizing their close similarity to mammalian retroviruses, Niewiadomska and Gifford have been sleuthing among the

genomes of reticuloendotheliosis viruses (REV) of birds. Sequences of these viruses have surprisingly also turned up within the genomes of a couple of large DNA viruses: fowlpox and gallid herpesvirus 2 (Marek's disease). Phylogenetically, REV history stretches back beyond the origins of echidnas in the mammalian lineage, more than 8 million years ago, but they only turned up in birds during the 1930s. How? Via human intervention. Further sleuthing in the literature led to just one archived sample of duck infectious anemia virus, which traced back to a series of experiments on a bird malaria isolate collected in Southeast Asia. There was a lot of interest in this newly discovered parasite, and samples were disseminated to five differ-

Continued on page 17



BIOMEDICINE

Skin Treatments

Epidermolysis bullosa is the term used to describe a group of inherited disorders that are characterized by severe blistering and skin fragility. Two recent papers show the beginnings of new therapeutic approaches. Dystrophic epidermolysis bullosa is associated with mutations in type VII collagen that result in blistering, deformities, and aggressive squamous cell carcinoma. Woodley *et al.* demonstrated that intravenously injected, recombinant type VII collagen was able to home to the region of wounds and restore expression for several weeks in two animal models. Junctional epidermolysis bullosa, in which the causative mutation may occur in the type XVII collagen gene, is corrected in some cells by a secondary mutation, leading to revertant mosaicism. Revertant keratinocytes should have significant potential as an autologous therapeutic agent. Gostynski *et al.* found that although colony-forming potential was high, revertant cells divided more slowly than wild-type cells. However, revertant cells were capable of engraftment and skin regeneration in a humanized mouse model if the cells were first grown as part of a skin equivalent on a plasma-based scaffold. — BJ

J. Invest. Dermatol. **133**, 1910; 10.1038/jid.2013.308 (2013).

Continued from page 15

ent laboratories. Unfortunately, the malaria samples had somehow become contaminated with REV, possibly during serial passage using mammalian blood or possibly by contact with the bêtes noires for pathogen spillovers—bats. The REV was then able to integrate into bonafide bird viruses, and thence into vaccine strains, and ineluctably became one of the hazards modern poultry have to face. — CA

PLOS Biol. **11**, e1001642 (2013).

MATERIALS SCIENCE

Doping by Diffusion

The electronic properties of bulk semiconductors can be improved by doping, the deliberate introduction of impurity atoms that increase the number or mobility of charge carriers. Usually, just doping the surface of a bulk semiconductor is sufficient, but for semiconductor nanocrystals, the entire particle needs to be doped to achieve the desired properties. Vlaskin *et al.* report on the doping of CdSe nanocrystals with Mn ions, a process that has proven especially difficult at the high levels desired to impart magnetic properties. Hot-injection synthesis of Mn-doped CdSe nanocrystals (where the particles rapidly form in solution from precursors) results in particles that are depleted in Cd²⁺ at the surface. The authors were able to incorporate Mn²⁺ ions uniformly by starting with CdSe seed nanocrystals, which were injected as a solution along with selenium into a solution of manganese acetate at ~300°C for times varying from seconds to several hours. This thermodynamically controlled process, which uniformly increased the size of the seeds, resulted in diffusion doping of the entire crystal without requiring Cd²⁺ ion ejection and allowed doping levels of Mn²⁺ as high as 20% to be achieved. — PDS

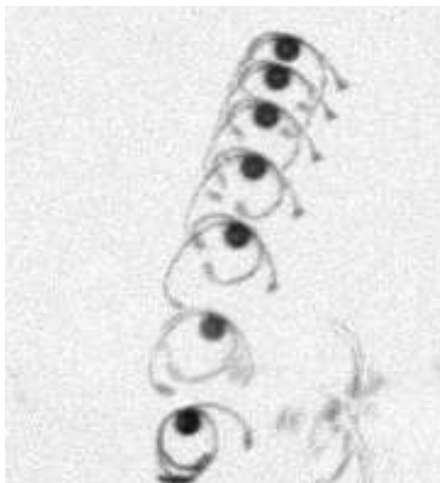
J. Am. Chem. Soc. **135**, 10.1021/ja4072207 (2013).

PLANT SCIENCE

Dessicated Dispersal

Equisetum plants (horsetail) have a lineage dating back to the Paleozoic, and these unusual vascular plants reproduce with spores. Marmottant *et al.* have now taken a closer look at how the spores get around. The spores have four long legs that, in humid conditions, are wrapped closely around the spore body, but as the relative humidity decreases, the legs straighten out. The change in shape is driven by the two-layer construction of the legs, with one layer having a greater tendency than the other to change volume in response to moisture, similar to the change in shape of old bimetallic thermostats in response to changes in temperature. The process

is reversible, with legs furling and unfurling as the humidity goes up and down. In the naturally moist habitat that *Equisetum* frequents, a shaft of bright sunshine or a dry breeze can effectively change the local humidity surrounding a spore. As the legs move, so moves the spore. Occasionally the legs get stuck on each other, and, when they get unstuck, the release of elastic energy



can propel the spore into a rather large leap. Whether crawling or leaping, repeated cycles of motility would increase the dispersion of these otherwise sedate plants. — PJH

Proc. R. Soc. B **280**, 10.1098/rspb.2013.1465 (2013).

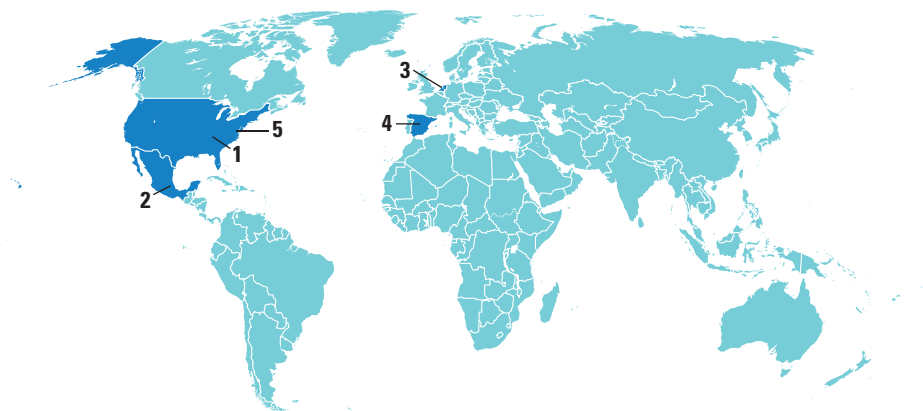
MICROBIOLOGY

Parasite Palmitoylation

The molecular mechanisms involved in the active invasion processes used by apicomplexan parasites such as *Toxoplasma gondii* to enter host cells are not well understood. Compounds that inhibit—or in some cases enhance—the infectivity of *T. gondii* parasites were recently identified in a chemical-genetic screen. How such small-molecule invasion enhancers might work, however, remains obscure. Child *et al.* have now been able to identify the molecular target of one class of small-molecule enhancers: a *Toxoplasma* enzyme, palmitoyl protein thioesterase-1 (TgPPT1). Inhibiting this thioesterase enhanced the invasive capacity of tachyzoites by increasing parasite motility and promoting secretion from micronemes, the parasite's invasion-associated organelles. TgPPT1 acts as a depalmitoylase, removing fatty acids that have been attached to proteins post-translationally. Thus, reversible palmitoylation within the parasite appears to play a key role in the invasion of host cells by *T. gondii*. — SMH

Nat. Chem. Biol. **9**, 651 (2013).

AROUND THE WORLD



Oak Ridge, Tennessee 1

National Laboratory to Cut Staff

Oak Ridge National Laboratory in Tennessee announced voluntary buyouts to pare up to 475 positions from its staff of 4450. The drawdown is the second in 3 years; if fully implemented, it would leave the lab with nearly 1000 fewer workers than it had in 2010.



Leaders. Oak Ridge Director Thom Mason (right) and Secretary of Energy Ernest Moniz (center).

Oak Ridge is one of 10 national labs run by the Department of Energy's (DOE's) Office of Science, whose budget this year fell 5% to \$4.632 billion as part of the automatic budget cuts known as sequestration. Oak Ridge is preparing for a second round of sequestration that will occur in 2014 unless Congress agrees on another way to reduce federal spending, says Oak Ridge Director Thom Mason.

"We've reached the point where people are telling me, 'I can't cut anymore because I've only got one expert in each of these different areas,'" Mason says. Directors of other DOE labs say they are also considering possible staff reductions. <http://scim.ag/ORNLCuts>

Pachuca, Mexico 2

Cholera Outbreak Puts Mexico on Alert

One person has died of cholera in Mexico, with 44 other cases reported in the state of Hidalgo in September. Thirty-three other suspected cases are awaiting laboratory confirmation. The Mexican Ministry of Health (SSa) has issued an alert instructing health authorities around the country to be on the lookout for the disease and to immediately report suspected cases to the ministry.

The outbreak is centered in the Huasteca region of Hidalgo, which, like many other parts of Mexico, suffered from torrential rain and disastrous flooding when Tropical Storm Manuel and Hurricane Ingrid hit both coasts simultaneously in mid-September. In an official statement, SSa said health care workers are fanning out across the region to screen households for cholera; deliver medication and training to local hospitals; and distribute disinfectants and prevention information, which includes washing your hands after using the bathroom and disinfecting uncooked foods. The last cholera outbreak in Mexico drew to a close in 2001, with only a handful of cases reported since.

Haarlem, the Netherlands 3

Flu Virologist Loses H5N1 Case

Virologist Ron Fouchier of Erasmus MC in Rotterdam, the Netherlands, has lost a court case against the Dutch government over a controversial study that led to the creation of an H5N1 flu virus that's transmissible between ferrets. On 20 September, a district court in Haarlem concluded that the government was right to order Fouchier to apply for an export permit before sending a manuscript about his work to *Science*



All that glitters. H5N1 viruses (in gold).

last year—a ruling that may complicate future flu work.

Fouchier's study was one of two H5N1 studies that caused an uproar in late 2011 because of their alleged potential for misuse by bioterrorists. The Dutch government asked Fouchier to apply for an export permit before submitting his paper (*Science*, 20 April 2012, p. 285); Fouchier did so under protest—and received the license—but Erasmus MC went to court over the case.

The district court rejected Fouchier's argument that the study fell under an exemption for basic research. Erasmus MC has not decided whether it will appeal.

<http://scim.ag/Fouchierfight>

Madrid 4

New Science Budget Disappoints Researchers

Spain plans to slightly increase its science budget next year following several years of drastic cuts. The national budget for civil research is to reach €5.633 billion in 2014, representing a 1.3% increase over last year. Of this, €2.250 billion will be distributed as competitive research grants and lump sums to public research institutes, a 6.1% increase over 2013. (As in previous years, the remainder will support companies with loans.)

But Spanish scientific societies, research centers, trade unions, and others have insisted that the survival of Spanish science requires bringing research spending back to its 2009 level by 2016, which would require an increase of €636 million a year. "The Spanish government has completely ignored the requests of the entire scientific society," says Amaya Moro-Martín, an astrophysicist who is a spokesperson of the researchers' organization Investigación Digna. The budget is now to be debated in parliament.

Science LIVE

Join us on Thursday, 10 October, at 3 p.m. EDT for a live chat on **predatory publishers and open access**. <http://scim.ag/science-live>

Washington, D.C. 5

Senate Ends Helium Saga

The U.S. Senate ended a protracted ping-pong match over the future of the helium market on 26 September, approving legislation that would allow the government to continue selling helium from a national reserve. The gas is coveted by scientists and high-tech companies alike. Many scientists who use helium in the lab will be relieved to see the matter settled. But a new law won't solve one problem, some say: Prices could stay relatively high for buyers of small quantities, stretching research budgets. The Senate's approval would clear the way for President Barack Obama to sign the bill.

NEWSMAKERS

Three Q's

Catherine "Cady"

Coleman is a chemist, former Air Force officer, flute player, scuba diver, and astronaut. And, most recently, Hollywood adviser. In 2011, while aboard the International Space Station, Coleman helped Sandra Bullock find her motivation as an astronaut set adrift after a collision with space debris destroys her shuttle. The new movie, *Gravity*, opens 4 October.



Coleman

Q: What did Bullock ask?

C.C.: We talked about a lot of things ... how you moved, how you kept yourself in one place. We talked about ... the emotional component of living someplace dangerous, and a place where you can be truly alone. That part of it is really challenging and hard, and this movie brings that home to me.

Q: How realistic is the movie?

C.C.: There are a lot of things that [the film does] really nicely—the look and the feel of living in low-Earth orbit, that isolation, and the specialness of having a view that not many people get to have. [But] there are a lot



Space jam. Astronaut Bullock is set adrift in *Gravity*.

Random Sample

A Panorama of Corals

Virtual divers, take a deep breath: An underwater world is at your fingertips. Last week, the Catlin Seaview Survey launched the Global Reef Record, a database of gorgeous high-resolution images of corals. The site (<http://www.globalreefrecord.org/>) will be a one-stop shop for images and data such as turbidity and water temperatures, says Ove Hoegh-Guldberg, director of the Brisbane, Australia-based Global Change Institute and chief scientist for the survey. "It's what I'm calling the world's largest stocktaking of corals in history."

Funded by international insurer Catlin Group Ltd., the survey uses a high-resolution camera that simultaneously takes images in three directions—right, left, and down—to make its panoramic coralscapes. So far, the survey—which began in September 2012 and will continue for 2 years—has recorded images of more than 32 reefs along Australia's Great Barrier Reef. Next up in 2014: the Coral Triangle, in the western Pacific Ocean. The goal is "to reveal the oceans to the world," says Richard Vevers, an advertising executive-turned-underwater-photographer who helped create the survey. One partner, Google Oceans, is already helping with that: In August, more than a billion virtual divers toured Google "street views" of the Great Barrier Reef.

Other partners include the National Oceanic and Atmospheric Administration and the Scripps Institution of Oceanography in San Diego, California. The team began a new effort last week in the reefs around Bermuda, hunting for signs of coral bleaching, a hallmark of coral mortality due to prolonged seawater heating. Next, researchers at Scripps will use an image algorithm that can detect low levels of bleaching not readily visible with traditional transects.



of coincidences—each is perhaps possible—but it's not probable that they would all happen on the same day, at the same time, or in the same order.

Q: What about space debris?

C.C.: The reason NASA doesn't make documentaries that keep people in their seats is because each of the risks in this movie is real, but we work really hard to mitigate those risks. There's a team in Colorado that tracks every piece of orbital debris over half an inch. ... These things are real, but we know how to deal with them.

FINDINGS

ID'ed: Culprit Behind Medieval Eruption

About 750 years ago, around 1257 or 1258 C.E., a powerful volcano erupted somewhere on Earth. That much of the story is written in polar ice cores: The amount of sulfur the volcano sent into the stratosphere was eight times as much as the Krakatau eruption in 1883 and twice as much as that of Tambora

in 1815. The climate impact in the Northern Hemisphere was pronounced: a cold summer, incessant rains, floods, and poor harvests, according to medieval records.

But identifying the volcano responsible has been tricky. Now, using geochemical, stratigraphical, and even historical data, a team of scientists has identified a likely culprit: Indonesia's Samalas volcano, on Lombok Island. *Babad Lombok*, Indonesian historical records written on palm leaves in Old Javanese, describe a catastrophic eruption of Samalas before the end of the 13th century that devastated surrounding villages with ash and fast-moving sweeps of hot rock and gas.

Studying outcrops and sediment analyses of these deposits, the researchers estimated the volume of ash erupted and the height of the eruption plume and reconstructed the original caldera topography—all pointing to an eruption of magnitude 7, making it one of the largest in the Holocene, the team reports this week in the *Proceedings of the National Academy of Sciences*. <http://scim.ag/Samalas eruption>



At work. Despite the shutdown, the Smithsonian's Carla Dove will continue her airstrike studies.

SCIENCE FUNDING

U.S. Shutdown Spares An 'Essential' Few

A bird identification expert might not seem like an employee essential to keeping the U.S. government functioning. But ornithologist Carla Dove was one of a select corps of federal scientists deemed important enough to be exempted from a sweeping government shutdown that began just after midnight on 1 October, paralyzing research funding agencies, shuttering a wide range of science projects, and sending home more than 800,000 federal employees.

"I'm getting prepared to be lonely," Dove said the day before the closure, noting that most of her colleagues at the Smithsonian Institution's National Museum of Natural History in Washington, D.C., wouldn't be allowed to work. "It will be me and 650,000 museum specimens."

The shutdown is the result of an epic stalemate between the Democrat-controlled Senate and the Republican-controlled House of Representatives. They could not agree on how to finance the government for the 2014 fiscal year, which began on Tuesday. It is the first shutdown since the winter of 1995–96, when an impasse over spending priorities prompted agency closures that lasted nearly a month. This time, however, the disagreement centers on efforts to undo the new U.S. health care law known as Obamacare.

The crisis came to a head this past week, as Senate Democrats four times rejected

bills passed by House Republicans to defund or delay Obamacare in exchange for funding the government for a few months at existing levels. Without such appropriations, agencies technically have no money to spend.

A shutdown doesn't mean that the entire government closes. It was largely business as usual at agencies deemed essential for public safety and national security, for instance, including the departments of Justice and Homeland Security.

But research agencies were hit hard. At the National Institutes of Health (NIH), 73% of its more than 18,600 employees were ordered to stay home. Although outside researchers could still submit grant applications through automated systems, NIH won't process them. And study sections won't meet to review pending applications.

The widely used PubMed database, which holds biomedical papers and abstracts, was not being updated, and the GenBank gene database wasn't taking new data. No new patients were admitted to the NIH Clinical Center, where more than 1400 studies are under way. The grants system also ground to a halt at the National Science Foundation (NSF), which spends 95% of its budget on research done by others. (Researchers who already had money in hand from both agencies could continue to draw funding.)

Every agency, however, has a list of employees that it says should keep working no matter what. At NSF, that "exempted" workforce amounted to just a few dozen people responsible for security, information systems, and overseeing its Antarctic program. NASA's few workers included those watching over the International Space Station. At NIH, more than 5000 employees were deemed critical to caring for patients and other tasks, including about 730 who were maintaining experiments in NIH's 1140 intramural laboratories.

One scientist on NIH's essential list is Al Singer, chief of the Experimental Immunology Branch at the National Cancer Institute. He reported to work on shutdown day to tend the lab's colony of 14,000 mice, who give birth to 1200 pups each week that must be genetically tested and culled. "There's no way this can stop," he tells *Science*. A veteran of the last shutdown, Singer worries morale will suffer. "It's a major disruption and people look at their job differently afterwards," he says.

In Boulder, Colorado, a skeleton crew of physicists and technicians is maintaining a cesium fountain clock that keeps official U.S. time accurate to 1 second every 100 million years, says Tom O'Brian, chief of the time and frequency division of the National Institute of Standards and Technology. The clock—"one of the most complex pieces of equipment in the world," he says—is essential to keeping GPS units, electrical power stations, and telecommunications networks in sync.

At the Smithsonian, Dove will be doing a job that her bosses deemed essential: trying to identify birds that have collided with aircraft. On Wednesday, she was expecting feathers and other samples taken from a helicopter crash last March. A contract with the Pentagon requires a quick turnaround. Fall is always her busy time, she adds, with seasonal migrations leading to up to 50 bird strikes a day. Overall, just 12% of some 6400 employees at the Smithsonian, which is two-thirds federally funded, were allowed to work.

How long Dove and other essential scientists will be working without their colleagues was unclear as *Science* went to press. Also unknown is whether, once the crisis is resolved, the furloughed workers will be paid for the missed time.

—JOCELYN KAISER, DAVID MALAKOFF, JEFFREY MERVIS, ELIZABETH PENNISI, KELLY SERVICE

CLIMATE SCIENCE

The IPCC Gains Confidence in Key Forecast

Last week's report from the Intergovernmental Panel on Climate Change (IPCC) contained a little-noticed scientific success story.

The latest assessment includes plenty of highly confident statements about how humans are messing with climate (see sidebar). But at first glance, it also suggests that in decades of study, scientists haven't made one whit of progress on the biggest question in climate: By how much will a doubling of atmospheric carbon dioxide (CO₂) levels warm the world? The range of "climate sensitivity" cited in the latest report is no different from the first official estimate in 1979—an eventual warming of 1.5°C to 4.5°C.

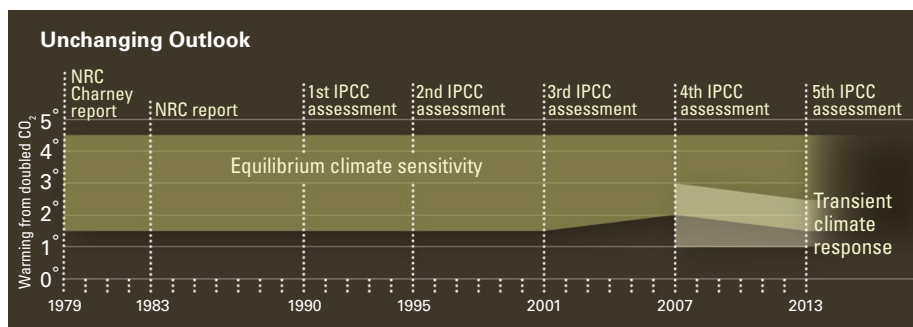
In fact, the new report presents the consensus range with far more certainty than ever before. It also brings a second sort of climate sensitivity measure to the fore, one better anchored in reality and of more use to policymakers. These advances gave Secretary-General Ban Ki-moon of the United Nations, a sponsor of the IPCC, one more reason for his rallying cry at last week's press conference: "The heat is on; we must act."

Back in 1979, the late Jule Charney of the Massachusetts Institute of Technology convened a committee for the U.S. National Academy of Sciences (NAS) to consider what effect, if any, increasing amounts of atmospheric CO₂ might be having on climate (*Science*, 13 August 2004, p. 932). One of the two U.S. groups modeling climate at the time told the committee that in their model, doubled atmospheric CO₂ warmed Earth by

2°C. The other group said its equally rudimentary model warmed 4°C after a doubling. Both figures represented the equilibrium climate sensitivity—the warming after the entire climate system had had time to adjust to the doubling.

Charney simply took 0.5°C as a not-unreasonable margin of error and came up with the now-iconic range for climate sen-

an e-mail. The vastly improved models now number in the dozens, and records of past climate also contribute—both those dating back millions of years and those for the past century. The report now assigns "high confidence" to climate sensitivity falling in the canonical range. And whereas the previous report could not exclude sensitivities even higher than 4.5°C, the new one finds that



Progress, really. Confidence has grown that equilibrium climate sensitivity falls within an unchanging broad range. Predictions of transient climate response—more useful to policymakers—have sharpened.

sitivity of 1.5°C to 4.5°C. It has endured through nearly 3 decades of reports, first from the NAS and then from the IPCC.

But even though the sensitivity numbers have stayed the same, the work behind them has advanced dramatically. "Our estimates are supported by actual data to an extent that was probably unimaginable in Charney's time," writes climate scientist and an IPCC report author Gabriele Hegerl of the University of Edinburgh in the United Kingdom in

sensitivities above 6°C are very unlikely. "Quite an advance," Hegerl writes.

The report also addresses the abstract quality of equilibrium sensitivity. "Equilibrium climate sensitivity is kind of an odd diagnostic, since it represents something that has never been, and will never be, observed in nature," writes modeler and IPCC author Gerald Meehl of the National Center for Atmospheric Research in Boulder, Colorado, in an e-mail.

A Stronger IPCC Report

Last week's fifth international climate assessment report, the first since 2007, took an already highly confident scientific consensus and substantially strengthened it. The Intergovernmental Panel on Climate Change (IPCC) revisited its three most fundamental conclusions from 2007:

Past warming. "Warming of the climate system is unequivocal." The last report reached the same conclusion.

Responsibility. "It is *extremely likely* that human influence has been the dominant cause of the observed warming since the mid-20th century." That statement supersedes 2007's "very likely."

Future heat. The world will likely get dangerously warm. Under all but the most draconian reductions in greenhouse gas emissions, it will warm more than 1.5°C to 2°C by the end of this century, much as the panel concluded in 2007. Two degrees of warming is now a widely, though informally, recognized "danger level" above which society would suffer serious consequences.

On other contentious points, the IPCC took a moderate line:

Rising sea level. Criticized in 2007 for underestimating how high warming would drive sea level, the IPCC this time forecast a rise of 40 to 60 centimeters by late in the century and a worst case of 1 meter by 2100. That's higher than in 2007 but far below the meter or two of sea-level rise that some expect.

Disappearing Arctic sea ice. The panel expects that if emissions continue to rise sharply, the Arctic Ocean will be mostly ice-free in summer before midcentury—50 years earlier than forecast in 2007. Arguments that summertime sea ice could be gone by 2030 lost out.

Extreme weather and climate. Humans have already contributed to an increase in the frequency and duration of heat waves and an intensification of heavy precipitation events, the panel found. More warming will not only "very likely" drive further such changes but will also likely intensify some tropical cyclones and deepen droughts. But the panel stopped well short of linking most every hurricane or drought to global warming.

—R. A. K.

Enter the transient climate response: the warming at the time CO₂—growing at 1% per year—has doubled (likely late this century). “We now realize that for the near term, the equilibrium climate sensitivity is not as useful as the transient climate response,” Hegerl notes. The transient response “can be estimated more robustly from the observed warming” than equilibrium sensitivity can be calculated, she writes. And the transient response is of far more use to policymakers; it can give them

an idea of how bad things are likely to get within a comprehensible time horizon.

Like climate sensitivity, the transient response is better constrained this time around. In the 2007 report, the transient response had a likely range of 1.1°C to 3.1°C. The latest report puts the likely range at 1°C to 2.5°C and says it is extremely unlikely to be above 3°C. “This makes a difference to forecasting warming,” says climate scientist Myles Allen of the University of Oxford in the United Kingdom.

Encouragingly, the range of transient response calculated from observations of past climate trends matches the range simulated by models. As a result, “we’re expressing greater confidence that future warming will fall in the spread of the models,” Allen says. “That has big political implications.” One big one: The world has already emitted more than half the CO₂ needed for a doubling that could bring the transient warming to dangerous levels well before the end of the century.

—RICHARD A. KERR

CLIMATE SCIENCE

For Researchers, IPCC Leaves a Deep Impression

Conservation biologist Terry Root was one of hundreds of volunteer authors on the 2001 report by the Intergovernmental Panel on Climate Change (IPCC). Root, of Stanford University in Palo Alto, California, came away with a sense of satisfaction, and something else: an idea for a major study to fill a gap she had noticed. Two years later, Root published a much-cited paper comparing 143 previous studies of how climate change can alter the life cycles and ranges of plants and animals.

Root isn’t the only scientist who has had their work reshaped by the IPCC, which last week issued the first part of its fifth report (see p. 23). Although scholars debate just how much influence the voluminous documents have had on the world’s policymakers—their main target audience—there’s little doubt about the deep stamp they’ve left on global science. Not only does the process make a major claim on the time of scientists in dozens of disciplines, it also spurs research collaborations, ties up years of expensive supercomputing time, and prompts a deluge of submissions to key journals.

As governments begin the next IPCC cycle, some planners are wondering whether the effort should be revamped. “The process takes too much time, tying up our best researchers,” says Karl Taylor, a climate scientist at the U.S. Department of Energy’s Lawrence Livermore National Laboratory in California.

Others say the investment is worthwhile. Michael Oppenheimer of Princeton University notes that the panel’s use of a multistage review process, fully documented online, provides a level of authority that makes researchers take notice of the report’s findings—and of the unresolved questions it highlights.

IPCC’s 2007 report, for example, said computer models’ predictions of sea-level

rise could be strengthened if missing details like dynamic ice sheet behavior were added to the simulations. Subsequently, Oppenheimer says, “several dozen papers poured out” to address the shortfall. A similar response occurred after the report suggested a 2°C rise in global temperature could increase the rate of species extinctions by 20% to 40%. “That

900 papers since 2007. “We wouldn’t have this level of participation [in CMIP] without the IPCC,” says Stanford University climate scientist Noah Diffenbaugh.

The IPCC’s high profile has inspired governments to devote more funding to modeling, says Bill Large, a manager at the National Center for Atmospheric Research in Boulder, Colorado. The United States has opened relatively new supercomputing centers in Wyoming and Tennessee. “It’s not clear these resources would have been forthcoming without the IPCC,” Large says.

The IPCC’s prestige drives researchers to finish work. To have a chance of being cited in last week’s report, researchers had to submit their papers to a journal by 31 July 2012. In the months before the deadline, *Nature Geoscience* received a surge of papers: about 170 per

month, or 24% above average. But there was a downside to the scramble, the editors noted in a recent editorial: papers not “as carefully thought through” as desired.

Such concerns are likely to get aired this month, when the IPCC’s member governments meet. One idea is to shift from comprehensive reports to smaller, more targeted ones, or to spread out the release dates of the three parts of the report, instead of packing them into a single 8-month period.

In the meantime, this year’s authors are beginning to relax. “It feels good to be done,” Vecchi says. But he hasn’t yet launched the proposed experiment with his new colleague. Why? “We’re still catching up on all the other things that piled up while we were working on the report.”

—ELI KINTISCH

IPCC by the Numbers*

- 859 authors and editors from 39 nations
- 2214 pages
- 41 climate models
- 2 million gigabytes of modeling data
- 9200 papers cited
- 54,677 comments

• *Working Group I report on climate science

[uncertainty] drove a lot of papers,” Root says. And this year, climate scientist Gabriel Vecchi found himself disagreeing with a co-author about the variability of the climate system. Soon, they began planning an experiment to resolve the matter, says Vecchi, who works at the Geophysical Fluid Dynamics Laboratory in Princeton, New Jersey.

Climate modeling also gets a unique boost from the report’s 6-year publication cycle. Although the IPCC sponsors no research itself, it catalyzes an international effort called the Coupled Model Intercomparison Project (CMIP), involving some 26 modeling teams. All do the same experiments, allowing comparisons. These “IPCC runs” can tie up supercomputers for a year before the reports are due—but have been used in more than



NEUROSCIENCE

Brain Stimulation Sparks 'Machiavellian' Choices

Imagine that you're playing a simple economic game, in which you receive a large chunk of money up front. You have to decide how much, if any, to give to another player who received little. The catch is that if you offend Player B by not sharing enough, she can shrink your stash.

You might assume that your decisions will emerge from a distinctive stew flavored by your personality, your upbringing, your moral code around sharing, and so on. That's all probably true, but a study published online in *Science* this week (<http://scim.ag/RuffrLPFC>) shows that a weak electrical current directed toward a patch of brain on the right side of your head can trump those influences. Depending on the direction of the current, participants in such a game shared more or less money, apparently becoming more or less sensitive to the social norm of being fair.

The study establishes a specific brain region, the right lateral prefrontal cortex (rLPFC), as a crucial nerve center for fairness, researchers say. It "provides a full leap forward in understanding where and how in the brain we attend to possible punishments as we contemplate our next moves," says Owen D. Jones of Vanderbilt University in Nashville, an expert in law and neuroscience.

People have played such money-sharing games countless times to help economics researchers probe issues of altruism, cooperation, and fairness. Economist Ernst Fehr of the University of Zurich in Switzerland, co-author on the new study, argues that in many cultures, a cultural norm of fairness—and a fear of retribution if that norm is violated—encourages people to split money equally. The researchers crafted their game to expose the workings of that norm. In a baseline round, Player B has no way to sanction Player

A for defying the norm; the latter therefore typically gives away only a little money. In the so-called punishment round, Player A faces a threat from Player B, who can expend her own funds to cause Player A's money to vanish. People typically share much more, 40% to 50%, in this case.

In the study, 63 female undergraduates—a sample chosen to minimize differences in variables such as sex and income—played the game for real money while a positive, negative, or sham (placebo) electrode was placed directly over their rLPFC. Brain-scanning studies by several groups, including the authors, had suggested that this area is involved in following social norms and in the urge to punish violators, says co-author Christian Ruff of the University of Zurich. But he, Fehr, and their colleagues wanted to find out if the rLPFC plays a causal role, or whether its activity simply correlates with such behavior. They hoped that transcranial direct current stimulation (tDCS) could do that, by making neurons in that area more or less excitable, depending on the current applied to it.

The team didn't find exactly what they expected: Turning up this brain region with tDCS "did not merely make people more norm-compliant in all situations," Ruff notes. During the baseline round, participants with a positively charged electrode—which boosted rLPFC activity—selfishly kept even more money to themselves than when the current was off, thus violating the norm. But once they faced a risk of punishment, the norm was reinforced, and they shared even

Social network. Stimulation of certain brain cells (*left*) influences how closely people follow a social norm of sharing.

more than they had during the sham trial. "The neural mechanism we identified seems Machiavellian, in that it allows participants to flexibly adjust their behavior to the presence or absence of punishment threats," Ruff says.

As people make choices, the brain weighs competing factors, Fehr explains. "It's a conflict—your self-interest is to keep more money, but your moral instinct is to give more away, and to obey the social norm." He speculates that "the brain stimulation changes the weights of the different motives." Neurophysiologist Michael Nitsche, a leader in tDCS studies at the University of Göttingen in Germany, calls the circuitry's flexible response "an important new mechanistic finding." (The women playing under a negatively charged electrode, which decreased activity in this brain region, made opposite choices, giving away more money in the baseline round but changing their behavior much less when facing possible punishment.)

Although manipulating their rLPFC apparently affected their decisions, the participants were unaware of this. Their conscious evaluations of the game, measured through questions such as how much anger Player B might feel at a certain response, remained the same as during sham stimulation. "It's a little scary," Fehr says.

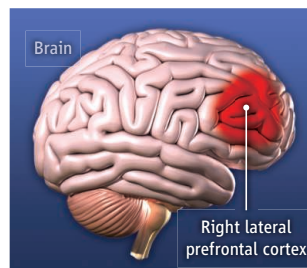
The experiment suggests that the brain regions involved in understanding a norm are "separately deployed" from the regions that choose whether to comply, Jones says. "This is a big step on the path to uncovering the brain phenomena by which people

choose to break the law."

Many evolutionary psychologists believe that norms emerge from individuals' own cost-benefit calculations, rather than being distinct features of societies, notes anthropologist Robert Boyd of Arizona State University, Tempe. This paper, by beginning to delineate specific neural mechanisms for conforming to norms, supports the idea that such behavior played a key role in our evolution, he says.

"We're a species who rose to prominence because of our ultrasociality," Jones agrees. "That would have been flatly unachievable, were it not for evolved brain mechanisms enabling us to create, attend to, and enforce social norms."

—ELIZABETH CULOTTA



EVOLUTION

Large-Scale Gene Comparisons Boost Tree of Life Studies

Emily Moriarty Lemmon will never forget how she struggled as a grad student to build a family tree for chorus frogs. To figure out how these sonorous amphibians are related to one another, she needed to assess as many DNA differences between species as she could, but time and money greatly limited the number of comparable sites she could identify and test. “It was really frustrating and kind of pointless,” Lemmon recalls. In the end, she could not assemble enough data to build a satisfactory chorus frog tree, or phylogeny.

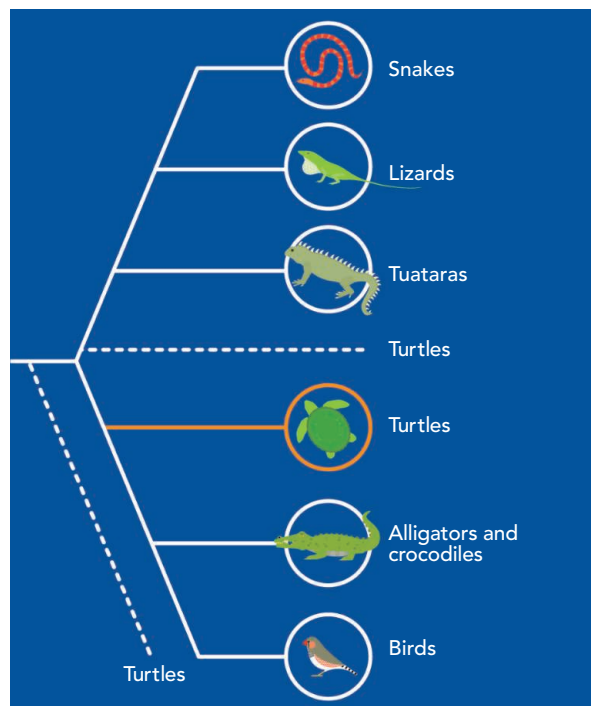
So in 2009, when she and her computer science-savvy husband, Alan Lemmon, began their faculty positions at Florida State University in Tallahassee, they decided to come up with a technique that would spare their students and others similar pain. They developed kits of genetic markers, identifiable in the DNA of many species, that provide a set of standard reference points for measuring genetic differences between species—and hence how closely they are related. Over the past 6 months, the couple embarked on a sort of promotional tour for the method at several major evolution meetings, sometimes wearing black T-shirts sporting their method’s name: anchored phylogeny.

Their technique and similar approaches relying on predetermined sets of markers found in the genomes of multiple species are streamlining the study of the tree of life. “It’s amazing,” says Chris Simon, a molecular systematist at the University of Connecticut, Storrs. “You can spend your time working on the data analysis rather than spending huge amounts of time working on data collection.” By making data collection for tree-building more comprehensive and faster—a matter of weeks rather than years—the new techniques promise to clarify our picture of the ancestry of familiar creatures. Already, one team pioneering the approach has redrawn the family tree of reptiles, showing that turtles are more closely related to crocodiles than to lizards.

Scientists originally drew family trees based on visible features—the number and placement of limbs or fins, or the shape of jawbones in fossilized remains, for example. Then genetics took over phylogenies,

with biologists determining species relatedness from how DNA sequences differed among species. But pulling out even a few genes or DNA regions that are appropriate for such comparisons used to take years. As a result, there’s often been too little data to resolve tricky branches of a tree, such as where the species in question are very distantly related or evolved very quickly. In theory, tree-builders can now simply sequence the whole genomes of multiple species and compare them, but that brute-force approach is still too expensive and complicated for many organisms other than microbes, with their simple genomes.

The strategy embraced by the Lemmons



Turtle triumph. A method using ultraconserved DNA shifted the putative turtle branches (dashed lines), placing turtles (orange) closer to crocodiles and birds than to lizards and snakes.

and others offers a middle ground: hundreds, even thousands of DNA regions to compare for a fraction of the cost of a whole genome. The key is identifying sequences common to many species that researchers can easily capture using specific probes—short pieces of DNA that home in on matching DNA. These shared sequences are sometimes too similar across species to help much with building a family tree, but they are typically

flanked by variable DNA that can be much more revealing.

Travis Glenn, an evolutionary biologist at the University of Georgia, and Brant Faircloth, now an evolutionary biologist at the University of California, Los Angeles, have focused on ultraconserved elements (UCEs), 100- to 200-base sections of DNA that are virtually the same across many vertebrates. Identical probes work in species separated by hundreds of millions of years of evolution. These probes fish out not just the UCEs but also flanking variable DNA. With sequence capture, “what you are sequencing is what you want ... and [you] can look at the same markers in a wren, a snake, and a turtle,” Faircloth says.

In early 2012, he and his colleagues demonstrated the technique on the placental mammal tree, showing that it placed multiple species on their correct branches. Later in the year, in *Biology Letters*, they published a UCE analysis that recast the reptile family tree, potentially resolving a longtime controversy by showing that turtles are more closely related to crocodiles and birds than to lizards and snakes. They have also refined the avian family tree, and in a just-published study of five rainforest birds, they showed that the technique can reveal the beginnings of new branchings, when species start to split.

The Lemmons use a different set of conserved markers. They didn’t think such highly uniform markers as UCEs were needed and so looked for sequences that were similar in specific groups of animals but not necessarily exactly the same. To come up with their first set, they compared the human genome with those of the chicken, green anole, Western clawed frog, and zebrafish, identifying 512 DNA sequences in common to all and existing as single copies in the genome. They then paid a company to make thousands of probes for a “vertebrate kit,” which targeted the DNA regions shared by the five species.

The probes work in many other vertebrates as well—including the Lemmons’ chorus frogs. By using the probes to fish out and compare several hundred regions of DNA, Emily Lemmon was finally able to build a credible family tree, she reported at an evolution meeting in Ottawa in 2012.

Since then, the Lemmons have built a kit that includes more probes from reptiles and amphibians and, together with collaborators

in Australia, they are pulling out DNA from all that continent's frogs, as well as many of its snakes and lizards. "They've got a very clean operating system that they've developed so that anybody can get plugged into a great assembly line process to get their data," says David Weisrock, an evolutionary biologist at the University of Kentucky in Lexington.

They have worked with Simon, of the University of Connecticut, to develop a kit for a group of insects, the Paraneoptera, which includes true bugs, lice, thrips, and cicadas. Simon hopes the effort will resolve the relationships among the several hundred cicada species she studies. There are also kits for beetles; Hymenoptera, the group that includes wasps, bees, and ants; annelids; and one in the works for plants.

Alan Lemmon, a theoretical evolutionary biologist, estimates that they now have 50 collaborators, with more inquiries coming in every week. Collaborators provide the DNA of organisms for which they want to create a tree, and the Lemmons design the



Phylogenetics family. Emily and Alan Lemmon want biologists to use their approach to building family trees.

probes, sequence the DNA, and do bioinformatics quality checks and preliminary analyses at cost (about \$175 per sample)—in exchange for co-authorship. In 2012, they processed 300 samples, and they expect to process 5000 a year by 2014. Some have criticized this as a "pay to play" approach, but Alan Lemmon emphasizes there's no profit made and says that the collaborators get a useful service. "We can get their feet wet without them having to spend a lot of resources," he says.

Faircloth and Glenn's UCEs are also gaining traction, as they and others use them to identify variable DNA in organ-

isms ranging from fish to primates and eventually, plants and invertebrates. They take a different approach to pushing out their technology, having created a website where the probe sequences, protocols, and software are free for all to use. So many other researchers have contracted one company, called MYcroarray, in Ann Arbor, Michigan, to put together

probes that the company just started offering several probe kits as catalog items.

As to which is better: "They're the Coke and Pepsi" of molecular phylogenetics, says Bryan Carstens, an evolutionary biologist at Ohio State University, Columbus.

Other researchers are coming up with their own flavors of sequence capture. The diversification is just what you'd expect from a successful innovation. Says R. Alexander Pyron, an evolutionary biologist at George Washington University in Washington, D.C.: "It's really light years ahead of what we were doing in the past."

—ELIZABETH PENNISI

BIOMEDICINE

NIH Seeks Better Database for Genetic Diagnosis

In 2011, Heidi Rehm, a molecular geneticist at the Harvard-affiliated Brigham and Women's Hospital in Boston, was asked to help physicians follow up on a prenatal ultrasound scan that showed a "nuchal translucency," a low-density area near the spinal cord of a fetus. It's viewed as a sign that the fetus might have a disfiguring condition called Noonan syndrome. So Rehm's lab did a DNA test, which came back positive for a gene variant listed in the lab's internal database as Noonan-related. The parents ended the pregnancy.

Many months later, however, the lab learned that the researcher who linked the variant to Noonan syndrome had concluded it was benign after all. But there was "no easy way to put that information in the public domain," Rehm says. So it had been set aside.

To make that less likely to happen in the future, the National Institutes of Health (NIH) last week awarded \$25 million for a new project called the Clinical Genome Resource, or ClinGen. The plan is to create a single reference point for data on medically important gene variants. Rehm, one of 10 researchers funded as part of the award, hopes the project will reduce uncertainties in genetic diagnosis.

She recently took part in an unpublished

experiment in which three clinical labs tested their prowess at making diagnoses from the same DNA sample. When they compared how they rated genes for medical significance, Rehm says, the labs disagreed on 20% of them.

According to Lisa Brooks, overseer of the project at NIH's National Human Genome Research Institute, there are now about 2000 databases on genes and diseases worldwide. Each lab concentrates mainly on its own work. ClinGen will scoop up clinically relevant information on gene variants from as many databases as will cooperate, review them, and share interpretations. The aim, Brooks says, is to create a "curated and annotated" collection of all medically relevant human gene variants. The ambitious effort builds on a preexisting public database called ClinVar at NIH's National Center for Biotechnology Information. ClinVar already contains more than 51,000 reported gene variants from 60 signed-up contributors. Johns Hopkins University in Baltimore, Maryland, heads the donor list with 23,642 entries. Rehm's lab is second, with 6996.

ClinGen is supposed to expand this data collection and improve its quality. (ClinVar

is not curated now.) One funded effort—including Rehm's lab; Robert Nussbaum at the University of California, San Francisco; and others—will develop standards and solicit genetic data and associated medical records from patients and doctors. Keeping personal information private while sharing data online will be a big challenge, Rehm says.

A second group will classify gene variants according to medical significance and call on specialist panels to make calls about specific cases—for example, to decide whether a variant is pathogenic or not. The third group will work on computerized data classification and release. One ambitious goal is to devise algorithms that predict the medical relevance of variants.

Sherri Bale, managing director of GeneDx in Gaithersburg, Maryland, who took part in the DNA interpretation experiment with Rehm, says that DNA testing companies like hers recognize the value of the project. "Without a curated database of variants, we are not going to be able to move into the whole-genome world," she says. "It has just got to happen."

—ELIOT MARSHALL



The Art of Eradicating Polio

The world is close to wiping out the poliovirus,
but Nigeria threatens to undo it all.
Muhammad Ali Pate is on a mission to change that

KADUNA AND KATSINA STATES, NIGERIA—

The boy, who looked to be about 16 years old, was alternately defiant and tearful. His father had left early that morning with strict instructions not to let the polio vaccinators inside. So when the vaccination team came to his house on a dusty street in a town in Kaduna state in northern Nigeria, he turned them away, and he wouldn't budge when they tried to push their way through the curtain that serves as a front door. When the team left, they marked the mud wall "RX" with chalk—code word for "noncompliant."

Now the boy was being summoned into the street by a very important man, at least judging from the TV cameras and the security detail surrounding him, although he was dressed simply, in a traditional white cotton robe.

I am Muhammad, the man said in Hausa, the language of northern Nigeria, resting his hand on the boy's shoulder. He said he wanted to know what was wrong and why the boy would not let the children be vaccinated. Then he settled in to talk in the sweltering midday sun.

For at least a half an hour, the man listened as the boy vented. The vaccinators had been rude, the boy said, insulting his mother as they tried to force their way in.

I would be angry, too, if someone insulted my mother, Muhammad replied.

Why do they bring only polio vaccine when we get no help with all our other problems? And are you going to force us to take it? the boy asked querulously.

No, it is your decision. I will not force you, the man assured him. But I hope that you will change your mind. Then he patiently explained that the vaccine is safe—he had vaccinated his own kids—and it would protect them from devastating paralysis. And also, that the world has a once-in-a-lifetime chance to eradicate polio—and the boy, and

Nigeria, should not stand in its way.

Then the boy's older brother, who had been listening from behind the curtain, emerged with one more question: Will you be responsible if the children are harmed? Yes, the man promised, and the brother brought the kids out to receive the polio drops. The crowd that had gathered around the house burst into applause.

And then the man, who at the time was Nigeria's minister of state for health, was back in the car as his heavily armed convoy sped off, lights flashing, sirens screaming,

"We passed the Rubicon when cases were so low in 2010. We knew we could do it, so there is no excuse not to do it. It is a moral imperative."

—MUHAMMAD ALI PATE

up the long straight highway that stretches from Abuja, the capital, through the increasingly desolate landscape of the north and then across the border into Niger.

It's a small victory, he confides later in the back seat of the Land Cruiser. But that's what it takes to eradicate polio in Nigeria. "There is science to polio eradication," he says. "But making it happen is art."

Colliding cultures

Muhammad Ali Pate has been trying to do what no one has been able to accomplish before him—finally drive the poliovirus from Nigeria, one of the last and most stubborn reservoirs in the world. The stakes are high: The outcome of the 25-year-and-counting effort to wipe the virus off the face of the earth rests in large part on the effort that Pate and his handpicked team have put together here in northern Nigeria.

You can't do it by fiat, Pate explains to me later during a 3-day car trip in mid-April through the north, where the virus is entrenched. Top down doesn't work in a coun-

try as complicated as Nigeria, an amalgam of colliding cultures and ethnic groups, with a discredited and powerless federal government and relentless insurgency and violence.

Instead, Pate, who hails from a Muslim village in the north, works from the ground up, persuading one boy in the streets of Kaduna North, or the next day paying courtesy calls to emirs at their palaces, or shaming local government officials who are misusing funds, or vaccinating kids in a nomadic community near the side of the road.

He didn't always think this way. He returned from the United States in 2008 with some decidedly Western views and a wonkish appreciation of health systems management. But Pate says he soon gained a respect for Nigeria's traditional culture and power structure—at about the same time he switched from wearing tailored suits to robes.

Pate is convinced that the virus can be dispatched from the country by the end of 2014, the new deadline set by the leaders of the Global Polio Eradication Initiative (GPEI), which has so far spent more than \$10 billion trying to eliminate the scourge of polio worldwide. More data, more money, and more troops are being poured into the effort than ever before, thanks to the Bill & Melinda Gates Foundation and other international partners and donors, who desperately want Nigeria to finish the job so the rest of the world can, too. And they have been betting big bucks that Pate, if anyone, can pull it off. He has come close before, getting cases down to a record low of 21 in 2010.

But in an unexpected move, Pate stunned the polio community when he resigned his Cabinet position on 24 July. Pate, who won't say why he quit, insists his commitment is undiminished. He says he will continue to lead the fight against polio as chairman of the influential Presidential Task Force on Polio Eradication, as long as Nigeria's President Goodluck Jonathan agrees. And he will continue his monthly visits to the critical northern areas to keep the pressure on local leaders and vaccinators.

CREDIT: HALLAH TASHKALMAH

Power of example. Muhammad Ali Pate gives oral polio vaccine to a Fulani child in Malumfashi in northern Nigeria.



Command central. From his office in Abuja, Pate has coordinated Nigeria's push to eliminate the poliovirus since 2008.

The top brass of the global eradication effort are optimistic that Pate has built a strong enough program that the country's recent progress can be sustained. "Pate has been absolutely crucial," writes Chris Maher, senior adviser for polio operations and research at the World Health Organization (WHO) in Geneva, who has worked extensively in Nigeria, in an e-mail. "But there is too much momentum in Nigeria for things to be easily derailed now," Maher adds: "His personal influence with the northern governors is unlikely to have diminished. If anything, he may actually end up being a more effective influencer from outside the government system."

Reinfecting the world

Since it started in 1988, GPEI, run by a partnership of WHO, the United Nations Children's Fund (UNICEF), the U.S. Centers for Disease Control and Prevention (CDC), Rotary International, and most recently, Gates, has driven polio cases globally down more than 99%. That last 1% has proved remarkably tough, but over the past 2 years, as global cases fell to an all-time low of 233 in 2012, the end has finally seemed in sight.

Nigeria stands in the way. Last year, it had more cases than any other country, and it was the only one of the last polio strongholds where cases went up. Nigeria, along with Pakistan and Afghanistan, is one of just three so-called endemic countries that have

never stopped transmission of the virus, even for a year. It is the only country in the world where all three types of the poliovirus are still circulating. It has had one of the largest and longest-running outbreaks of vaccine-derived poliovirus type 2. And of the three holdout countries, it tends to be the one that keeps GPEI officials up at night.

Nigeria has spawned more outbreaks in previously polio-free countries than the other two combined, earning its reputation as the country that reinfects the world each time the wild virus is almost gone. The latest such outbreak began earlier this year in Somalia and Kenya.

It's not that the virus circulating in Nigeria is any more dangerous. The problem lies in the lousy rates of routine immunization across a large swath of Africa, which leave huge numbers of kids vulnerable whenever the virus jumps the border from another country. By contrast, Pakistan's neighbors India and China have erected a high "wall of immunity" through strong and continuing vaccination campaigns.

As in the other infected countries, polio in Nigeria is a disease of the poor and disenfranchised. By 2005, polio had been dispatched from the relatively wealthier southern half of the country, which is mostly Christian. It took up refuge in the Muslim north, which has some of the most abysmal health and development indicators in the world. More than half of the population lives

in grinding poverty. According to recent UNICEF data, routine immunization rates in some parts of the north are as low as 13%. Many people there have no access to toilets and clean water, maternal and child mortality rates are off the charts, and diarrhea remains one of the country's biggest childhood killers.

The Human Development Index ranks Nigeria 153rd out of 187 countries; Transparency International places it among the more corrupt countries in the world. Understandably, there is no love lost between the poor of the North and the government they feel has abandoned them.

So when vaccination teams come around with nothing to offer but drops of oral polio vaccine (OPV), many people are suspicious and fall prey to rumors that the vaccine is

contaminated with the AIDS virus or infertility drugs, part of a Western plot to decimate the Muslim population. Rumors and misinformation reached such a frenzy in 2003 and 2004 that four northern states banned all polio vaccination outright, sending cases soaring to 1122 by 2006.

The challenges now are as great as ever. This year, the antipolio vaccination movement has resurged, with opponents circulating CDs and texting people in advance of campaigns, warning of the supposed dangers of the vaccine. The country's violence and terrorism took on a chilling new dimension with the murder of 10 polio workers in Kano in February.

That's why Pate is on the road for 3 days in April, as he has been every month since October 2012, visiting the worst places and using his signature brand of diplomacy to try to turn things around. The grueling trip shows, stop by stop, how he has again managed to bring cases down in 2013; it also gives a taste of the political and social complexities of working in a region that is so critical to the outcome of the global eradication initiative itself.

On the road: Saturday, 13 April

EN ROUTE FROM ABUJA TO KADUNA STATE:

The first thing you notice about Pate are his eyes: Wide set in a round face, they have such dark circles that they appear bruised. He looks perpetually tired. He is soft-spoken,

CREDIT: ALAMY

with a kind voice, and despite his many years in the West, speaks English with a heavy Hausa accent.

When he picks me up at the Rockview Hotel in Abuja, where about half the guests seem to be aid workers or U.N. employees, he is wearing a plain white cotton robe, as he will throughout the trip. His fula, the traditional round cap, is next to him on the seat. His black socks and lace-up shoes add an oddly Western touch.

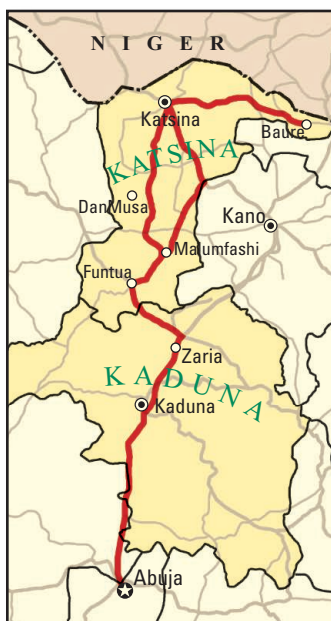
We are going to Kaduna and Katsina states, which last year had about 40% of the polio cases in the country and where, despite the country's stepped-up effort, vaccinators are still missing too many kids. As we set out, Pate talks about the murders in Kano, another of the high-risk states. At about 9 a.m. on 8 February, gunmen on motorcycles stormed two clinics in rapid succession, killing 10 health workers and one client, all women. The vaccinators had just assembled before heading out for their "mop-up" day, when they try to find and vaccinate kids missed during the previous 3-day door-to-door campaign.

Violence and terrorism in Nigeria are nothing new. The Islamist insurgency is so intense that the president in May declared a state of emergency in three northern states

and sent in troops. Schools have been burned, students murdered, villagers massacred. Aid workers have not been immune. In August 2011, 23 were killed and scores injured when the Islamist insurgent group Boko Haram bombed U.N. headquarters in Abuja. But polio workers had never before been directly targeted. No one has claimed responsibility.

President Jonathan and Pate immediately condemned the killings, and Pate visited the

The journey



Campaign route. In mid-April, Pate drove hundreds of kilometers and made dozens of stops across the Nigerian states of Kaduna and Katsina, where polio is entrenched and resistance to vaccination is high.

grieving families in Kano. Vaccination rounds continued as scheduled the next month. "We are not going to stop or the militants will have gotten what they wanted, so we forge ahead," he says. Asked whether he feels in personal danger, Pate concedes, "sometimes, yes. I am a visible target for those who don't want the program to succeed. Boko Haram is not against polio per se, but it is a high-profile program, high visibility. If you want to attack the government, it is an easy target."

KADUNA STATE BORDER: As we cross the border into Kaduna state, the convoy is flagged down by a dozen or so cheering men by the side of the road, emissaries of the governor and deputy governor, who will join us for the day. Donning his fula, Pate jumps out of the car to thank the welcoming committee, and the political drama begins.

Meeting with senior state officials a few minutes later, Pate explains: "I am here as the chairman of the Presidential Task Force on Polio Eradication to encourage you to see every child is immunized, that this be the best round of 2013. When I looked at the results [from last month], there has been a lot of progress, but in Kaduna state, there is lots of room for improvement." Then it is off to see the rounds.

The convoy stops in the middle of a narrow street in the market in Kaduna South, and Pate is out, working the crowd. The ramshackle market, with its stalls stacked high with grains, fruits, and vegetables, is one of the "fixed posts" to vaccinate kids who aren't home—or whose parents say they aren't—when the teams go door-to-door.

Pate is there to see the polio survivors, wearing their bright green vests emblazoned with "Kick Polio out of Nigeria forever," courtesy of Rotary International, which has volunteers in all the endemic countries and has so far contributed more than \$1 billion to the cause. The men, whose legs are paralyzed, pull themselves along the street with their strong arms, using flip-flops as shoes on their hands. They smile broadly when the minister meets them, proudly showing Pate a shiny blue motorcycle they built as part of a rehabilitation program to give the survivors jobs. "Polio has left you with paralysis of the body, but your minds are active," Pate says as he thanks them for their help.

At a time when polio has become all but



Vaccine advocates. Legs paralyzed by the disease, polio survivors play an important role in persuading reluctant parents to let their children be immunized.

invisible and few people have seen how it can ravage the body, the survivors are some of the program's most effective advocates. "Parents don't want their kids to be like that," Pate tells me when we are back in the car.

KADUNA NORTH TOWN:

There is no shade at the next stop, where Pate persuades the boy and his brother to let the kids be vaccinated, and the heat has easily topped 105. The car feels almost incredibly cool when we climb back in.

Pate is pleased. "This is important diplomacy." He concedes that in the grand scheme of things, one or two missed kids won't make a difference—and you certainly can't spend half an hour with each one. "But this family was contaminating the atmosphere," he says. And besides, he adds, he wanted to demonstrate the art of persuasion for the vaccinators.

"I was very conciliatory. I feel the pain of poor people," Pate tells me. "They need so much. Why is it only polio we offer, why do we keep going back?" They may want vaccine against a deadly measles outbreak, or a new road that the government has promised. Refusing polio vaccine is the only leverage they have.

That's why one of Pate's signature programs is called Saving One Million Lives. The goal is to avert 1 million deaths by 2015 by offering basic services such as routine immunization, rehydration salts and zinc for diarrhea, bed nets for malaria, and antenatal and newborn care—"Who could be against maternal and child health?"—and also polio vaccination. "It is not either or, polio or other health needs. We do both. It works very well."

MEETING HALL, KADUNA NORTH DISTRICT:

After opening prayers, Pate begins to speak, commending the district chairman, the equivalent of a mayor in the United States, and assembled officials for their efforts. It's a tough spot to work, he concedes; rumors are rife, refusals are high, the large migrant population is hard to reach.

"Boko Haram is not against polio per se, but it is a high-profile program, high visibility. If you want to attack the government, it is an easy target."

—MUHAMMAD ALI PATE

But then the gloves come off. Despite a "surge" of about 150 additional staff members from WHO, CDC, and UNICEF for this critical last stage (*Science*, 3 August 2012, p. 514), the district missed some 30% of kids in the last round. "With all these boots on the ground, why is there no progress in Kaduna North and Kaduna South?" Pate asks.

"I don't want Nigeria to be forever remembered as the

last country in the world with polio—it is an issue of shame. Even in Nigeria, there will be a last state and a last local government. I don't want Kaduna North to be the last local government."

It's a drama he will enact repeatedly over the next couple of days—praise and then pressure. It's the only way he can work in the districts, which are autonomous and have long been the Achilles' heel of the program, he says. "I can't sanction them," he says. "So I have to cajole and influence."

"It is up to you to see the funds are used prudently," says Pate, who has learned that the chairman has not yet released the district's contribution to the campaign and has not been attending the requisite planning meetings. "If money is diverted ... you will have blood on your hands."

KADUNA STATE GOVERNMENT LODGE:

"Very few people upset me. I don't think there are difficult people, just different people. But he upsets me," Pate says over lunch at the government lodge. He is talking about Haruna Kaita, a professor of pharmacy at

Ahmadu Bello University in Zaria and one of what Pate calls the "pseudoscientists" who have spread rumors about the polio vaccine. In 2004, Kaita announced he had analyzed samples of OPV and found traces of estradiol and other contaminants, feeding into the furor that led to the vaccination ban of 2003 and 2004.

The ban was lifted only after intense national and international lobbying, with then-President Olusegun Obasanjo assembling several delegations to test the vaccine, and GPEI providing assurances that only vaccine made in Indonesia, a Muslim country, would be used in Nigeria.

In January this year, Kaita teamed up with an influential cleric and made a CD, widely distributed, repeating many of the earlier assertions. Kaita alleged that the polio vaccine "contain[s] birth control and birth defect-causing substances ... [and] that children could contract other diseases through the [vaccine], it could be cancer, HIV, or mad cow disease ..." The cleric, Ibrahim Ahmad Aliyu, said, according to a translation: "Forceful oral polio vaccinations are an American-planned genocide against the Muslim populations in Nigeria."

When Pate first came back to Nigeria, he debated the skeptics. "I thought I could reason with them scientist to scientist," he says. But since the February killings, Pate's stance has hardened. "They have gone too far," he says. He blames the opponents for indirectly inciting violence. Now, Pate says, instead of reasoning with them, "We will engage them one by one."

With Kaita, Pate says he has some leverage: "If he is a professor at a public university, he is a government employee." In late

April, Kaita released a two-part statement carried in the newspapers "clarifying" his position "following attempts in high and low places to ridicule my painstakingly cultivated reputation." He recognized "the existence of the polio epidemic and the mechanism of administering appropriate vaccines to prevent its spread in Nigeria" and backed off some of his more inflammatory claims. But he also complained that agents of the State Security Service had visited his home several times



Winning hearts and minds. A UNICEF community volunteer in Kano teaches a father about polio. In the end, he agrees to let his seven children be vaccinated.

and the police had called him to Abuja in an effort to intimidate him.

EVENING MEETING, KADUNA NORTH: The sun is down, but the heat is still insufferable in the packed room where the day's vaccination efforts are being reviewed. Even Pate is sweating.

One by one, the supervisors report how many houses their vaccination teams visited, how many kids were in each, the number immunized, the number missed, why, and so on. One team leader says the women have no power. Another says some mothers want to vaccinate their kids but tell her that their husbands will divorce them if they do. It is hard to know how much is the truth, how much excuses. But it is clear many kids were missed, and many teams will be sent back into the field to mop up the next day. Similar meetings take place across the northern states after each vaccination day, part of a massive effort Pate has put in place to pinpoint trouble spots, where more effort is needed.

HOTEL SEVENTEEN, KADUNA: It's late when we reach the hotel, an incongruously modern building that just opened and is still working out the kinks. As we wait for my room to be readied, Pate tells me the circuitous route that brought him to polio.

The oldest of 10 kids, Pate, 45, grew up in a small town in Bauchi state. His father is Fulani, the largest nomadic pastoralist group in West and Central Africa; his mother is part Fulani and part Hausa, the main ethnic group in northern Nigeria. His father was the first in the family to graduate from university, and Pate remembers him telling him as a boy: "I can't give you wealth or cattle. All I can give you is an education. You have to go to school, that is your path."

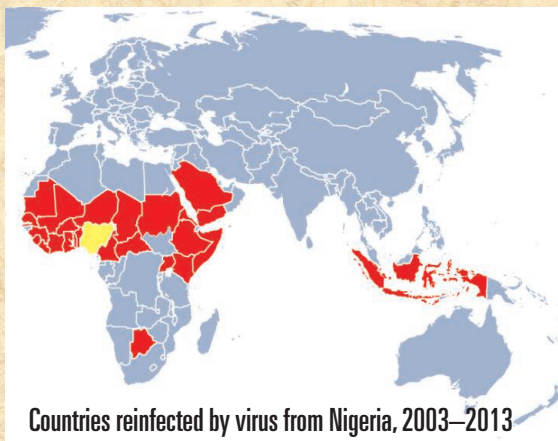
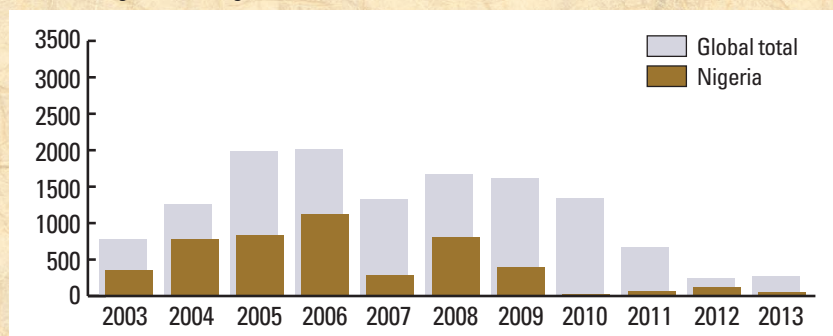
Pate took the advice to heart—as did his siblings, who have multiple advanced degrees among them—putting himself through college and medical school at Ahmadu Bello University in Zaria, Nigeria's ancient center of learning. He was restless—"my nomadic genes," he says—and wildly ambitious.

He landed a job as medical officer for the British Medical Research Council in The Gambia in 1993, then in 1995 went on to a residency in internal medicine at Howard University in Washington, D.C., followed by a fellowship in infectious disease at the University of Rochester in New York. Along the way he decided clinical work was "too reductionist." So while in Rochester he earned a master's in health systems management long distance from the London School of Hygiene & Tropical Medicine. Recruited to the World Bank in

SOURCE: WHO

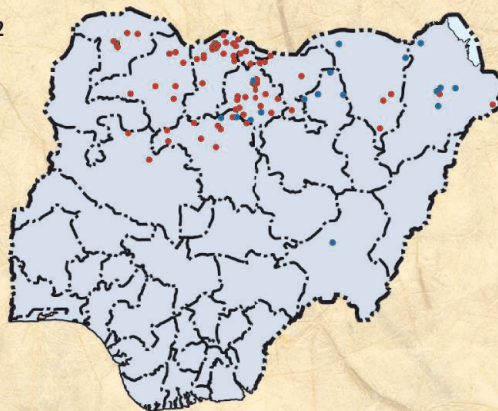
Global Threat

Polio cases, global and Nigeria, 2003–2013

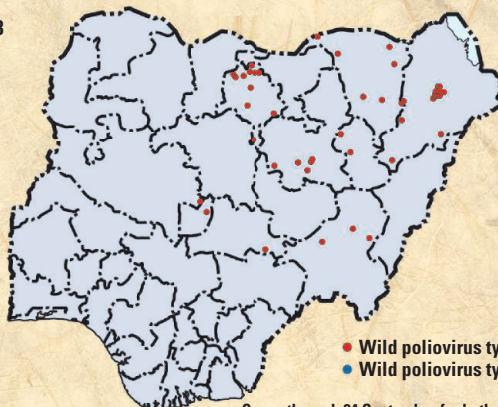


Linchpin. Nigeria is critical to the global effort to eradicate polio. It is just one of three countries, along with Afghanistan and Pakistan, where transmission has never been stopped. Since 2003, Nigeria has accounted for a significant share of cases worldwide, and virus originating there sparked outbreaks in 25 previously polio-free countries.

2012



2013



Cases through 24 September for both years

Polio in Nigeria

Progress. Under Nigeria's new emergency eradication plan, polio cases in the country have dropped about 45% from this time last year. Nigeria had 122 polio cases total in 2012; so far this year there have been 47, compared with 90 this time last year. Cases have shifted to the eastern half of the country, where the insurgency is intense and it is difficult to reach children during polio vaccination campaigns. There have been no cases of type 3 polio, showing gains against one of the two remaining wild serotypes.

2000, he worked his way up the ranks, ending up as country sector coordinator for human development in the East Asia/Pacific region in 2006. While at the bank, “I got the itch again,” he says, so he commuted from D.C. to Duke University in North Carolina on the weekends and earned a master’s in business administration.

In 2008, he got a call: Then-President Umaru Yar’Adua wanted to see his resume.

Nigeria had just been roundly condemned by the World Health Assembly for derailing the global eradication program. That year, Nigeria had some 800 cases. Humiliated, the president wanted to clean house. He offered Pate a job as CEO of the country’s National Primary Health Care Development Agency, the equivalent of the U.S. CDC, in October. There was no question. “I wanted to be a player,” Pate recalls. In November, after 15 years abroad, Pate was on the job in Abuja.

Two years later, polio cases had dropped to 21, the lowest ever. In 2011, the president appointed Pate the minister of state for health. With Pate’s attention diverted by his broader portfolio, cases began to climb, setting off alarm bells within the international community. By October 2012, Pate was back in charge, this time as chairman of the Presidential Task Force on Polio Eradication, and on the road.

On the road: Sunday, 14 April

THE PALACE OF THE EMIR OF ZARIA, KADUNA: The gate to the emir’s palace is a bright mosaic of greens, reds, blues, and golds, a stark contrast to the mud architecture of the rest of the old city.

Resplendent in an intricately embroidered, cream-colored robe and gauzy tufted headdress, the emir receives Pate on his red throne while his councilors sit cross-legged on the floor. After prayers, Pate offers his condolences for the recent death of the emir’s older brother. The emir, like other traditional leaders across the north, is a big supporter of the polio program, and Pate is full of praise and gratitude.

It’s the same drill the next day when Pate meets with the emir of Malumfashi. “You see the drama,” Pate says later of the pageantry, the elaborate robes, and the effusive praise. You have to be an artist to move between Nigeria’s traditional and modern worlds, he adds. “And for a long time the community will remember that the government, the

minister came to them. And he is here for polio. That is the influence that is so vital to the global effort.”

“I used to have a different view of the emirs. I thought sometimes our traditions were holding us back,” he tells me. That changed soon after his return when he went to a meeting called by a district chairman and almost no one was there. When he went to the emir’s palace, it was packed. “That made clear the dysfunction of the [district] system,” he says. “The district officials have the money, legal authority to tax. But they have no moral authority. The emirs have moral authority and a formidable network that you ignore at your own peril.”

Pate had another revelation soon after his return, when he decided to ditch his expensive suits for traditional garb. “I hadn’t worn a robe since ’95 when I got married. I came back here and wore suits. But then I realized I had to be part of the culture.” And besides, he says, the suits were way too hot.

EN ROUTE FROM ZARIA TO KATSINA STATE:

Pate is something of a data geek. During the long car ride, he recalls having lunch with Bill Gates a couple of years ago. “He asked really tough questions” about our data. “I had questions, too, I felt like I was flying blind.”

Gates brought in the Seattle group Global Good to develop a model to assess population immunity, or what proportion of the population is adequately vaccinated against polio. With foundation support, Nigeria introduced geographic information systems and global positioning systems, and satellite mapping found thousands of settlements no one knew existed. It turned out that polio cases were increasingly concentrated along the borders between states and districts, where everyone thought it was someone else’s responsibility, and along nomadic routes. Soon, digital maps replaced vaccinators’ hand-drawn plans.

Gates bankrolled the spiffy new Emergency Operations Center in Abuja, which now has offshoots in several high-risk states. For the first time now, the partner agencies

work in the same building, where data feed in from the districts and states. “Now we have data so we can make decisions in real time,” Pate exults.

As we are talking, the convoy pulls over at a boisterous nomadic settlement near the side of the road in Malumfashi. Pate jumps

out of the car and launches into Fulani, one of his native tongues. The crowd is thrilled as Pate vaccinates a baby and then good-naturedly lets himself be vaccinated. Laughing, he declines the candy given to the kids after their drops.

He is pleased to see the encounter broadcast later on TV.

KATSINA STATE GOVERNMENT LODGE: At dinner, Pate is tired, the circles under his eyes even more pronounced. He runs his hands across his face and close-cropped hair, then takes off his shoes and rubs his feet. Over chicken and fried rice, he tells me what keeps him on the road each month.

“We passed the Rubicon when cases were so low in 2010,” he adds. “We knew we could do it, so there is no excuse not to do it. It is a moral imperative.” And beyond Nigeria, “eradicating polio will be huge for the global health community, like going to the moon. It will unleash a huge momentum.”

It is 10:30 when the protocol chief finds us a suitable motel and Pate quits for the day.

On the road: Monday, 15 April

KATSINA DISTRICT: “We are in a difficult area,” Pate says the next morning as we begin our long last day in Katsina, Nigeria’s barren, northernmost state. Polio opposition is strong in this area; Kaita is from here, and the CD is circulating widely.

Pate meets briefly with the governor, then heads to the big event of the day: a ribbon-cutting ceremony for seven new buildings at the Federal Medical Centre in Katsina. Pate is the guest of honor, along with the emirs of Katsina and Daura, whose guards in their bright red and green turbans stand out among the sea of light-colored robes and who jostle with Pate’s nattily clad security detail. The guards with their leather whips sit at the emirs’ feet. Pate’s crew, along with federal police armed with AK-47s, spread out among the crowd.

Pate cuts the ribbon for a new laundry room that holds two new washing machines for the 515-bed hospital and the new intern housing, named the Muhammad Ali Pate Officers Quarters.

Then the convoy departs for a dozen far-flung districts—from Baure in the north to Funtua in the south. At each of the stops, Pate tells the local officials that he and 457 other scientists from around the world have just signed a declaration supporting GPEI’s new plan to wipe out the virus by 2018. Nigeria should not be backwards, he says.



Half a continent away. A young girl receives polio vaccine drops at a school in Mogadishu, where a new outbreak is raging, seeded by virus from Nigeria.

Pate and I part ways at 6 p.m. He is heading back to Abuja. I am staying in the north to observe vaccination campaigns. He insists I keep a car, a driver, and an armed guard. "It is not that dangerous," he assures me. "But people sometimes take advantage of foreigners."

Outbreak

On 9 May, the inevitable happened. A case of wild polio was confirmed in a 32-month-old girl near Mogadishu—the first there since 2007. Genetic analysis showed the virus came from Nigeria. A week later, another case was reported in Kenya in the Dadaab refugee camp near the Somalia border, the first wild case in that country in almost 2 years. Just when cases globally were at a record low, Nigeria had once again reinfected the Horn of Africa. By the end of September, cases in the Horn had soared to 191, more than double the number in Nigeria, Pakistan, and Afghanistan combined. The outbreak, spreading fast because the conflict has left so many kids unvaccinated, is a devastating blow and a tough test of GPEI's recently announced plan to stamp out any new outbreak within 120 days. This

time, they won't, program officials conceded in September.

Nigeria itself seems back on track. The program took a hit in March after the killings in Kano, when vaccination coverage stagnated or declined in some places. The influential Polio Eradication Independent Monitoring Board, which has often been scathing in its reviews, in May 2013 gave the country high marks, noting that "[t]he pace of improvement in Nigeria's Polio Programme over the last six months has been greater than at any other point in its history." Cases are down 45% from this time last year.

In the light of these successes, Pate's abrupt resignation as health minister on 24 July was met with an outpouring of praise and regret. "Pate has made a fantastic contribution ... and it's disappointing to see him move on from his position as Minister of State for Health," wrote Michael Galway, Gates's point person for Nigeria, in an e-mail. "But I feel confident the program will continue to move in the right direction."

Speaking on the phone on 21 August from his house outside Washington, D.C.,

where his wife and six kids now live, Pate declined to say why he quit. "I want to avoid speculation. ... I feel the time for the transition had come, it was the right time in a way that doesn't hurt the program."

Pate, who is an adjunct professor at Duke University, will likely take a new position advising the Gates Foundation. He says there's no reason to worry that the eradication will lose momentum. He points out there is a strong team in place, led by the executive director of the primary health care agency, the position Pate held when he first began to turn polio around.

"It is not only one person" he says. "It is the role one plays on the team. I can continue in that role even though I am not minister and help to complete eradication." He expects to remain closely involved in the campaign and plans to spend time each month in Nigeria.

Indeed, the day we spoke on the phone he was flying back to Abuja for another road trip to the areas where polio cases are now centered and the insurgency remains intense. Since our April visit, Kaduna and Katsina states have remained polio-free.

—LESLIE ROBERTS



LETTERS

edited by Jennifer Sills

NextGenVOICES

Results: Work-Life Balance

What one change would most improve work-life balance for scientists? In July, we asked young scientists to send us their solutions to this pervasive challenge. We heard from more than 200 readers. A sample of the best responses can be found below. To allow for as many voices as possible, in some cases we have printed excerpts of longer submissions (indicated by ellipses) and lightly copyedited original text for clarity. To read the complete versions, as well as many more, go to <http://scim.ag/NextGen8Results>. Follow *Science's* NextGen VOICES survey on Twitter with the hashtag #NextGenSci.

Submit Now: Enduring Ideas

Add your voice to *Science*! Our new NextGen VOICES survey is now open:

What recent discovery in your field will still be remembered 200 years from now? Why?

To submit, go to <http://scim.ag/NextGen9>

Deadline for submissions is 15 November. A selection of the best responses will be published in the 3 January 2014 issue of *Science*. Submissions should be 250 words or less. Anonymous submissions will not be considered. Please submit only once.

situation of low salaries for Chinese scientists. Almost no real increase in scientists' salaries is in sharp contrast to the rapid rise in China's housing prices and cost of living during the past decade. The poor salary system thus is pushing scientists to spend much more time working rather than being with family. To get extra income in China, many scientists are busy attending various meetings, establishing or maintaining close relations (guanxi) with government officials, indiscriminately applying for grants from different government agencies, and anxiously producing more and more papers.... More time on work means less time on life. The work-life imbalance puts Chinese scientists under high pressure. A sharp increase in scientists' salaries, I think, will most improve work-life balance for scientists, especially for young scientists in China....

FENGBO LI

State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Sericulture Research Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, 310021, China. E-mail: fengboli@gmail.com

AGE RESTRICTIONS WHEN

applying for grants or positions should be eliminated. Each of us has different personal circumstances that result in us taking more or less time to complete a Ph.D./postdoc or to apply for a group leader position. Having to plan each single step of your life is not living....

ANA LOZANO

Max F. Perutz Laboratories, University of Vienna, 1030, Vienna, Austria. E-mail: ana.lozano@univie.ac.at

TO IMPROVE WORK-LIFE BALANCE IN THE SCIENCES, a cultural shift is necessary that provides more space for creativity and places less emphasis on efficiency for efficiency's sake.... For early-career scientists, creative intellectual space can be hard to come by. Short-term postdoctoral positions com-

NextGen Speaks

RECESS! DO YOU REMEMBER THE UTTER JOY of running with reckless abandon while grinning unabashedly from ear to ear? As adults, we may not be able to recapture the simple joy and innocence of childhood playtime, but we can at least partake in physical activity to give ourselves a healthy break from daily responsibilities. To improve work-life balance and overall well-being, exercise during working hours should not be merely tolerated, it should be expected. We've done the research. Nothing else comes close to bestowing the broad benefits of a solid exercise routine. We can protect ourselves against aging-related cognitive decline, prolong the vivacity of our immune systems, strengthen our bones, and empower our mitochondria, as well as reduce our risk of cardiovascular diseases, diabetes, neurodegenerative diseases, and certain cancers. We've gathered the evidence, now let us set an example. Let us not

be the obese nutrition scientist or the oncologist who sneaks a cigarette; let us be the ones who deal with stressful lives by building our physical strength and mental fortitude through fitness. And let us not feel guilty for doing so. No longer should we have to sneak out of lab like thieves in the night to get in an hour's swim or feel obligated to stay late to make up for time "lost" at a lunchtime yoga class. Principal investigators, I call on you to value healthy lifestyles enough to insist that your employees and trainees make time to exercise during the day!

KELLY DOWNING

Stanford Cardiovascular Institute, Department of Vascular Surgery, Stanford University, Stanford, CA 94305, USA. E-mail: downing.kelly@gmail.com

"THOSE WHO RESEARCH ATOMIC BOMBS don't earn as much as those who sell tea eggs." This Chinese saying originated in the early 1980s, but still prevails nowadays in the Chinese scientific community. It ironically describes the





Science communication
then and now

49



Overcoming jet
lag

52



combined with increasingly vast amounts of data and journal articles to keep up with, and expectations to produce many articles oneself leave little room for messing around with truly novel research ideas (or even some false starts that might prove helpful in the future), let alone spending time with family and friends. How can we make a cultural shift toward giving ourselves more space for creativity in the workplace, and consider time spent away from work a critical way to “recharge”? One concrete proposal would be to shift toward longer-term (5-year) postdocs and grant cycles. This would give early-career scientists more flexibility to pursue original ideas and to take time for home life, exercise, even full nights of sleep. These healthy habits could then carry through to later career stages. Author’s note: I am writing this late at night on my daughter’s birthday, with less than 6 hours of sleep ahead of me before I travel tomorrow....

ANNE J. METEVIER

Institute for Scientist and Engineer Educators, University of California, Santa Cruz, Santa Cruz, CA 95064, USA and Department of Physics and Astronomy, Sonoma State University, Rohnert Park, CA 94928, USA. E-mail: ajmetevier@gmail.com

MAKE “NO” PART OF YOUR VOCABULARY. Especially as a young scientist, I was eager to prove I could handle everything (from publishing my papers to organizing social events) until I realized I could not if I also wanted a life. Then I found out that tasks can be broken down into three categories: Something is either important to me, or to someone else, or no one really cares.... Setting priorities helps to keep a work-life balance....



MARTINE J. VAN DER PLOEG

Soil Physics and Land Management Group, Soil Science Centre, Wageningen University, 6700 AA, Wageningen, Netherlands. E-mail: martine.vanderploeg@wur.nl

CHILDCARE. THE SINGLE BIGGEST LIMITATION on my ability to work late and flex around



subjects’ and colleagues’ schedules is the absolute requirement that I leave the building between 4:00 and 5:00 p.m. to go pick up my daughter from day care.... If I had a childcare solution close to my budget, I could put in longer hours and get more done. For reference, I am a male scientist.... This is no longer a female issue; it is a universal issue. My university does have an affiliated childcare center, but it has a waiting list of 2 years or more. That doesn’t work so well for new postdocs and faculty members who move.



ALIK WIDGE

Division of Neurotherapeutics, Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA 02129, USA and Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. E-mail: awidge@mit.edu

...WORKING IN SCIENCE MEANS MORE THAN just research: For many motivated scientists, it also involves organizing meetings (e.g., Ph.D. conferences organized by Ph.D. students), participating in science outreach (school visits and teaching in courses), and communication (blogging and tweeting). Yet,



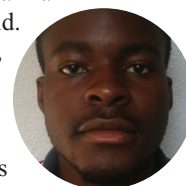
these activities are generally not reflected in science metrics, and for early-career scientists are not always assessed in fellowship or grant applications either. Thus, many of us consider nonresearch activities to be more like “hobbies.” Accordingly, such “extracurricular” projects are conducted in our free time, following already strenuous working hours. Therefore, a more nuanced evaluation of what we do, and a greater appreciation (academic and monetary) of nonresearch activities would encourage scientists to consider them as part of their work and thus allocate their time accordingly, which would ultimately result in an improved work-life balance.

ORSOLYA SYMMONS

Developmental Biology Unit, European Molecular Biology Laboratory, 69117, Heidelberg, Germany. E-mail: symmons@embl.de

INTRODUCING INSTITUTIONS for medical specialization

(and other essential specializations) in the developing world. In developing countries, such as most countries in Africa, scientists have to spend many years abroad and away from their families just to specialize. Some crucial specializations (such as in surgery) are very extensive and require several years.... Most scientists achieve further education at the expense of their wives and kids. This has given a bad name to such scientists; they are now seen as irresponsible husbands who hide behind books....



HAGAI MAGAI

College of Medicine, University of Malawi, Private Bag 360, Chichiri, Blantyre, Malawi. E-mail: hymagai@medcol.mw

IT IS MY FIRM BELIEF THAT THE THING THAT will most improve work-life balance for all scientists is ethical treatment in laboratories. Too often, we hear stories in our respective universities and companies of unethical treatment of students, professors, scientists, and researchers.... These ugly monsters raise their heads in the form of low salaries that require inhumane work hours as well as looking the other way when research integrity requires you to stand up and say



“this is unacceptable.” Unfortunately, many scientists are too scared to be whistleblowers in these situations, as it will affect their future career prospects.... With an improved ethical set of values in laboratories, we will surely see an increase in productivity and happiness among scientists.

K. CHRISTIAN KEMP

Department of Chemistry, Center for Superfunctional Materials, Pohang University of Science and Technology, Pohang 790-784, Korea. E-mail: ckemp@postech.ac.kr

...KEEPING A BALANCE BETWEEN WORK AND private life is all about managing your time. We all have exactly the same 24 hours a day, so scientists should be able to just work efficiently and go home early, right? Wrong. Nature knows neither schedules nor the concept of normal working hours.



Cells keep growing, animals need their daily treatment, and the protein purification column is simply not running as it should. The solution is increasing efficiency through teamwork. Life

in the lab can be lonely when you are the only one responsible for a project. However, when scientists, usually with different backgrounds, come together, a synergistic process starts and new ideas come up.... The biggest hindrance is unclear authorships and responsibilities.... However, I think that the increased efficiency in the lab and therefore more work-life balance would be worth the effort of trying to work more as the team we already are.

DENISE OROZCO

German Center for Neurodegenerative Diseases (DZNE), 80336, Munich, Germany and The International Max Planck Research School for Molecular and Cellular Life Sciences (IMPRS-LS), 82152 Martinsried/Munich, Germany. E-mail: denise.orozco@dzne.lmu.de

IN MY EXPERIENCE, MOST SENIOR RESEARCHERS still firmly believe that in order to be successful, you have to spend all your time doing research. As a consequence, younger researchers are spending inordinate hours in the office,



trying to live up to this view. More hours do not, however, mean a higher output, as tired researchers are less productive and less creative. Moreover, many people

are more productive at home than in the office. I think academic institutions should focus more on providing researchers with the skill set to increase their productivity, instead of just (implicitly) demanding more

and more hours. That way you can have a healthy work-life balance while still producing good quality research.

VINET COETZEE

Department of Genetics, University of Pretoria, Hatfield, Pretoria, 0028, South Africa. E-mail: vinet.coetzee@up.ac.za

...ONE OBSTACLE I'VE NOTICED LIVING IN a foreign country—and looking to move to another foreign country for a different position—is how unaccommodating some positions are for people with families.... Postdoctoral positions are sometimes set up with very little to offer in terms of health-care benefits and financial aid going toward childcare costs (which would be a given for citizens). A lot of postdoctoral positions given to foreigners appear to be offered with the “get in, get out” attitude and are simply not (socially) designed for people who have extra, maybe complicated needs (like kids)!...



ASHLEY J. RUITER

Max Planck Institute for Astrophysics, Garching bei München, 85748, Bavaria, Germany. E-mail: ajr@mpa-garching.mpg.de

FOR MANY SCIENTISTS, A HUGE PROPORTION of available time is spent on administrative and regulatory tasks that do not increase the quality or safety of research.



Furthermore, grant funding generally does not cover salary for administrative personnel to handle these non-study-specific tasks. Reducing administrative and regulatory burden to a more reasonable level—for example, in the oversight of human and animal studies—would be a huge step in making scientists' workloads more manageable.

ERIN J. WAMSLEY

Department of Psychiatry, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA. E-mail: EWamsley@bidmc.harvard.edu

QUITE SIMPLY: MORE SUPPORT IN DAILY household chores. Let the scientists spend the little free time they have with their family, instead of constantly having to clean, cook, do laundry, and drive kids around. Having a family should not come as an extra burden for the scientist, nor should it unequivocally mean that the scientist in question



will have to reduce his/her amount of time spent in the lab. They should either be paid more to afford this kind of help, or there should be a network of nannies and cleaners available to scientists to help them around the house. The German Christiane Nüsslein-Volhard foundation, for example, gives promising young female scientists monthly financial grants to pay for assistance in household chores and additional childcare—this type of initiative should become the norm, not an exception.

MORGANE BOONE

Lab for Medical Biotechnology, Department of Biochemistry and Microbiology, Inflammation Research Center, VIB - Ghent University, 9052 Zwijnaarde-Gent, Belgium. E-mail: morgane.boone@ugent.be

ONE OF MANY CHANGES THAT COULD BE made to improve work-life balance for scientists, especially the ones working as academics, is by reforming the way in which the academic reward system is carried out. It is time to reevaluate the real effectiveness of current practices, such as publication impact factor metrics, in achieving scholarly goals such as excellence. The pressure to publish is immense, but the quality of output does not often reflect the extent of reflexivity, self-critique, and insight that could be achieved through careful and diligent scholarly thinking. Output “quantity” in itself



is no measure of quality or excellence—real impact entails so much more. Genuinely acknowledging and rewarding these other key impact factors, such as societal engagement, teaching excellence, and input and deliberation at key meetings and conference participation could go a long way toward balancing the demands of academic output to publish alone, thus lessening the pressure to spend endless weekends and vacation time writing for quantity rather than quality.

CAROLINA ADLER

Institute for Environmental Decisions, Department of Earth Systems Science, ETH Zurich, Zurich, 8092, Switzerland. E-mail: carolina.adler@env.ethz.ch

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

PHYSICS AND BIOLOGY

From Matter to Self-Organizing Life

Arne Traulsen

Writing a book that reaches from elementary particle physics to natural selection is an ambitious task—either it becomes a too-*Short History of Nearly Everything*, or we learn to see the world through someone's personal New Kind of Science (1, 2). In his treatise *From Strange Simplicity to Complex Familiarity*, which he has worked on for 15 years, the Nobelist Manfred Eigen does not approach either of these apparent attractors. Instead, Eigen (Max Planck Institute for Biophysical Chemistry) aims to integrate current scientific knowledge from different fields to show that evolution is a physical process based on clear physical laws. But he also anticipates the criticism of the late Ernst Mayr, noting "Asking for a physical law behind Darwin's principle does not imply an attempt to make the whole process of evolution 'calculable.'" From the first pages, you can feel Eigen's elation for the natural sciences. He writes about elementary principles of physics with a young scientist's enthusiasm for his own field—not being an expert in particle physics, he simply states "I trust these people."

The book weaves together five chapters ("Matter and Energy," "Energy and Entropy," "Entropy and Information," "Information and Complexity," and "Complexity and Self-Organisation"), each organized into ten questions. Some of the questions are of the kind that kids would ask: "How many trees make a wood?" or "How large is zero?" But most kids will not understand the answers that Eigen offers. He supposes that the reader has a solid general education in the natural sciences, tacitly assuming familiarity with concepts from statistical mechanics, quantum mechanics, biochemistry, and genetics. But after technically demanding parts, the author always comes back to basic principles, allowing even readers having a less solid background to easily follow the main line of thought. They will find additional help in the appendices, written by Eigen and other

experts, which provide some insight into the most important technical issues. (One appendix courageously presents an entirely verbal characterization of phase transitions.)

Eigen argues that physical laws govern chemistry. Chemistry leads to the combinatorial possibilities of molecular biology. This complexity allows the emergence of evolution and the origin of life. In light of such a chain of causalities, it is difficult to argue what makes life possible. Cosmology must set the stage for the basic chemistry of life, but much more is needed for life to emerge: "Life is based on physics, but its ultimate outcome is of a sophistication that transcends anything we can describe by any known physical law." Eigen contends that what we need to tackle such emergent behavior is the physics of information, and he fills the book with beautiful examples from this field, which may at first sight seem to be a bit detached from the world of classical physics.

For decades, physicists have been trying to understand the relation of physics to life (3). As Eigen highlights, they have long been struggling to accept Darwin's

principle of natural selection, some of them favoring Lamarckism instead. Maybe part of this struggle reflects the limits of thinking in terms of equilibria and paths toward them, which is at the basis of many classical theories in physics and also in biology. The theory of systems far from equilibrium developed by Eigen's generation allows us to address such problems from an entirely new perspective (4). This approach is probably, as the author claims, a necessity: "Evolutionary optimisation could never occur under conditions near to equilibrium."

The book offers a joyful but not shallow route through the complications that arise

on the way from elementary particles to complex forms of life. However, Eigen only touches on some additional complications: If evolving entities can interact, then the fitness landscape (which provides the basis for evolution) may itself be a dynamic product of the interactions (5). In this way, evolutionary dynamics can lead to eternal cycles

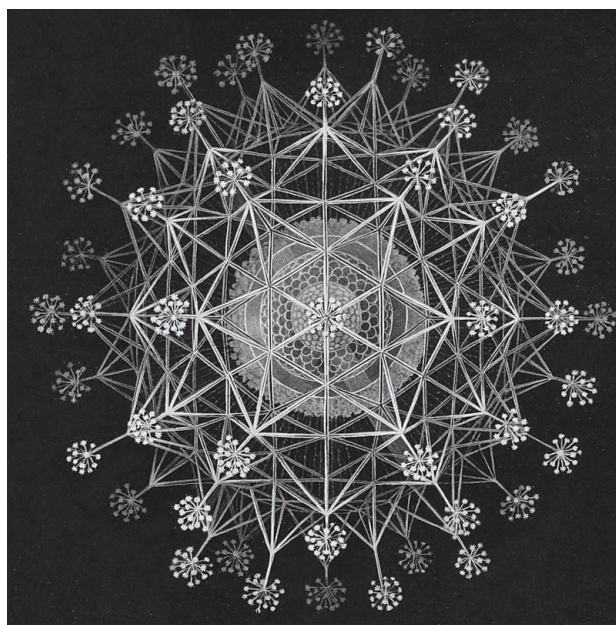
(6), which would only be halted by effects not covered by basic mathematical models of evolution.

The author enriches the book with many personal stories, included "just for fun." Reading about encounters with Werner Heisenberg, Paul Dirac, Motoo Kimura, and many others nicely complements the book's technical parts. My personal favorite is the story of the student Lars Onsager introducing himself to Peter Debye, his subsequent Ph.D. advisor, with the words "Are you Professor Debye? Do you know that your theory is wrong?" Maybe interactions between brilliant people—Debye earned the Nobel Prize in chemistry in 1936, Onsager in 1968—do not always follow the usual conventions.

From Strange Simplicity to Complex Familiarity includes Eigen's thoughts on two of the most mind-boggling questions in science: the origins of the universe and of life. Such philosophically deep issues are at the heart of many religions, but Eigen demarks a clear boundary between science and religion and joins those asking for mutual respect (7). He sees science as "a cooperative endeavour of humans" that tries to unravel the laws of nature. Like evolution, it never comes to a halt, and we will always find more

From Strange Simplicity to Complex Familiarity
A Treatise on Matter, Information, Life, and Thought

by Manfred Eigen
Oxford University Press,
Oxford, 2013. 754 pp. \$225,
£125. ISBN 9780198570219.



Evolved symmetry. Ernst Haeckel's depiction of the radiolarian *Sagenostella stellata*.

to explore by adapting to what is there. On the last page of the text, Eigen announces a planned second volume of his own endeavor, in which he will consider “life, thought and human culture.” I look forward to reading it—with the hope that this time we do not have to wait for 15 years.

References

1. B. Bryson, *A Short History of Nearly Everything* (Doubleday, London, 2003).
2. S. Wolfram, *A New Kind of Science* (Wolfram Media, Champaign, IL, 2002).
3. E. Schrödinger, *What Is Life?* (Cambridge Univ. Press, Cambridge, 1944).
4. N. Goldenfeld, C. Woese, *Annu. Rev. Condens. Matter Phys.* **2**, 375 (2011).
5. M. A. Nowak, K. Sigmund, *Science* **303**, 793 (2004).
6. B. Sinervo, C. M. Lively, *Nature* **380**, 240 (1996).
7. F. De Waal, *The Bonobo and the Atheist: In Search of Humanism Among the Primates* (Norton, New York, 2013).
8. E. Haeckel, *Kunstformen der Natur* (Bibliographisches Institut, Leipzig, 1904).

10.1126/science.1243730

RESOURCES

Energizing Consensus

Saleem H. Ali

The convergence of science and policy in the 2012 U.S. election was particularly evident in the realm of energy supply and demand. Polling in four swing states conducted by the American Council on Renewable Energy indicated that energy policies affected the choice of a majority of voters: 66% in Colorado, 60% in Virginia, 58% in Iowa, and 57% in Ohio (*1*). The renewed political salience of energy in the United States is the starting point for Michael Levi's *Power Surge*, which aims to reconcile the debate between fossil fuel enthusiasts and their renewable energy detractors. Levi rejects long-standing claims that the only way forward is to choose between starkly contrasting paths (*2*). Much of his narrative revolves around environmental and social conflicts that are evident in the United States, particularly around hydraulic fracturing (or fracking) and other unconventional energy-harnessing technologies.

Levi, a physicist who turned to policy analysis at the Council on Foreign Relations, argues convincingly that there is a place for both fossil fuels and renewable energy

The reviewer is at the Sustainable Minerals Institute, University of Queensland, Brisbane, QLD 4072, Australia, and the Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT 05401, USA. E-mail: saleem.ali@uvm.edu



in America's power portfolio. Written for an informed general audience, his account tends to simplify issues with anecdotes. But it proves far more nuanced and less ideologically driven than other popular books on the topic, such as Daniel Yergin's *The Quest* (*3*). Whereas writers like Yergin downplay environmentalist concerns when arguing that fossil fuel resources are far from depleted, Levi grapples with them, particularly the climate change aspects. He tries to pragmatically reconcile scientifically cogent concerns about climate change with a risk-based approach to transition fuels such as natural gas.

Despite the author's earlier work and expertise, he largely neglects nuclear power, offering only some passing references to its “wild card” status. Here he could have provided a more innovative account by considering developments in research on breeder reactors and thorium fuel opportunities. While Levi argues for embracing both traditional fossil fuels and new clean energy, he doesn't offer science-based analyses of biofuels or their place in the debates on renewable energy sources. I also missed discussions of the potential for algae-based fuels (*4*) and the use of nanotechnology in synthesizing fuel molecules.

Surprisingly for a trade publication, the book lacks any diagrams, graphs, or images. By summarizing data, these could have given more rigor to Levi's arguments while also improving public understanding of the complexities of energy technologies. In this regard, readers—especially those having a background in science—will find Vaclav Smil's books on energy policy (*5*, *6*) more multifaceted and informative.

In *Power Surge*, Levi relies considerably on itinerant field observations he gathered during travels across the shale-oil fields and coal country of the United States. Interviews with key decision-makers, including senior

executives at energy companies and international diplomats, provide empirical texture to his text. Throughout the book, Levi focuses on the most politically contested topics around energy, as well as those areas that most acutely affect consumers. For example, he highlights the centrality of the automobile in American fuel consumption and lifestyles by devoting an entire chapter to “The car of the future.” There, he skillfully weaves together conceptual and theoretical insights from broader literature and links them to the topic, as in a discussion of the Jevons paradox—the observation by 19th-century economist William Stanley Jevons

that, because of latent demand, greater efficiency can lead to increased consumption of resources. Levi deftly explains the flaw in applying this observation to driving behavior (as is often done by environmentalists): The biggest cost of driving is the value of people's time, so increased car use falls far short of negating the gains

from improved efficiency. Yet one would still like to find more about cases in which the paradox may well be applicable, particularly in the context of new urban development and power consumption. Levi largely neglects ways of countering the paradox and the work of dominant research centers in this field, such as the Rocky Mountain Institute.

Overall, *Power Surge* offers a highly readable account of a complex topic. Abandoning polarization, it opens the door for further scientific conversations to consider the possibilities of consensus on energy development strategies—a discourse desperately needed if we are to overcome political inertia. Levi demonstrates great breadth of knowledge and engagement with policies. The book would have benefited from additional details. Perhaps those could be included in an expanded edition.

References

1. www.acore.org/news-media/press-releases/2715-swing-state-polls-show-that-voters-support-clean-renewable-energy.
2. A. B. Lovins, *Foreign Aff.* **55**, 65 (1976).
3. D. Yergin, *The Quest: Energy, Security, and the Remaking of the Modern World* (Penguin, New York, 2011).
4. R. H. Wijffels, M. J. Barbosa, *Science* **329**, 796 (2010).
5. V. Smil, *Energy Myths and Realities: Bringing Science to the Energy Policy Debate* (AEI Press, Lanham, MD, 2010).
6. V. Smil, *Energy Transitions: History, Requirements, Prospects* (Praeger, Santa Barbara, CA, 2010).

10.1126/science.1243171

CREDIT: HAL BERGMAN/GETTY IMAGES

LAND USE

Managing Forests and Fire in Changing Climates

S. L. Stephens,^{1*} J. K. Agee,² P. Z. Fulé,³ M. P. North,⁴ W. H. Romme,⁵ T. W. Swetnam,⁶ M. G. Turner⁷

With projected climate change, we expect to face much more forest fire in the coming decades. Policy-makers are challenged not to categorize all fires as destructive to ecosystems simply because they have long flame lengths and kill most of the trees within the fire boundary. Ecological context matters: In some ecosystems, high-severity regimes are appropriate, but climate change may modify these fire regimes and ecosystems as well. Some undesirable impacts may be avoided or reduced through global strategies, as well as distinct strategies based on a forest's historical fire regime.

¹University of California, Berkeley, CA 94720, USA. ²University of Washington, Seattle, WA 98195, USA. ³Northern Arizona University, Flagstaff, AZ 86011, USA. ⁴U.S. Department of Agriculture Forest Service, Pacific Southwest Research Station, Davis, CA 95618, USA. ⁵Colorado State University, Fort Collins, CO 80523, USA. ⁶University of Arizona, Tucson, AZ 85721, USA. ⁷University of Wisconsin, Madison, WI 53706, USA.

*Corresponding author. E-mail: sstephens@berkeley.edu

Fire regimes are commonly characterized by burn frequency and severity within a given area. Severity is often estimated as the proportion of overstory trees killed by fire. In general, as frequency increases, fuels have less time to accumulate, reducing intensity and subsequent tree mortality. However, a great deal of variation occurs even within fire regime types (1). The spatial scale and patch-size distribution of different severity classes are key in assessing whether fire regimes have changed over time and whether changes maintain or compromise forest ecosystems.

Globally, fire frequency and severity vary among forest types. Essentially all fires have high-severity effects, where most of the trees are killed, at some spatial scale and patch size. The critical issue is whether tree mortality patch sizes (and their temporal and spatial frequency) allow recovery of the same or similar vegetation types. If high-severity patch sizes are too large, microclimates and regeneration mechanisms (e.g., seed abun-

Policy focused on fire suppression only delays the inevitable.

dance and dispersal) can limit tree reestablishment (see the figure). Large high-severity patches may produce vegetation type changes, especially in forests adapted to frequent, low- to moderate-severity fire regimes or in forests that lack in situ propagule sources. Introduced species, such as nonnative grasses, also may alter forest fire regimes and lead to changes in vegetation type (2).

Changing fire severity is at the heart of ecological debates about historically high-frequency, low- to moderate-severity fire regimes, such as ponderosa pine (*Pinus ponderosa*) and semiarid mixed-conifer forests. A central concern is whether high-severity patches in wildfires are too large, which results in undesirable ecosystem changes (see the figure). Rising temperatures, related drought stresses, and increased fuel loads are driving high-severity patches to extraordinary sizes in some areas (3).

In contrast, forests adapted to low-frequency, high-severity regimes such as Rocky



Historical forest fire regimes. (Top) Mixed-conifer forest in northern California with fuels accumulated from a century of fire suppression (left), mature surviving and regenerating trees in an area that had been mechanically thinned to reduce fuels and residues either removed or burned (center, 10 years after the 2002 Cone Fire), and an adjacent untreated area lacking live seed trees, now dominated by shrubs (right, 10 years after the Cone Fire). (Bottom) Lodgepole

pine forests in Greater Yellowstone (left) regenerated abundantly from the canopy seedbank after the stand-replacing 1988 Yellowstone Fires (center, 15 years postfire), but regeneration was greatly reduced in forests of comparable age and serotiny after the 2000 Glade Fire, which was followed by summer drought 1 year after the burn (right, 10 years postfire). Forests are within 4 km of each other at each site.

Mountain lodgepole pine (*Pinus contorta* var. *latifolia*) have evolved to regenerate after large, high-severity events. Seed banks stored in tree crowns survive even the highest-severity fires and are released shortly after the fire ends. If seeds germinate in open conditions conducive to relatively high growth rates, a new forest can become established in a few decades (see the figure). Other species can regenerate a new crown from one burned by fire because of dormant buds.

Future Fire Under Changing Climates

With projected climate warming (4), forests around the globe will likely undergo major landscape-scale vegetation changes in coming decades. In some areas, plant productivity may decline to a point where fire will become less frequent (5). In more productive areas, fire regimes may shift from being mostly climate-controlled (top-down) to mostly fuel-controlled (bottom-up) (6). In both cases, slow vegetation change may be abruptly accelerated by a change in fire regime driven by novel climatic conditions.

Increased frequency and size of large, severe forest fires are expected in Australia, the Mediterranean Basin, Canada, Russia, and the United States (3, 7, 8). In the western United States, increased frequency and size of fires is associated with increased temperatures, earlier spring snow melt, and longer fire seasons (9)—mechanisms that are applicable to other regions of the world.

Trends and projections of climate and fire responses suggest that new strategies to mitigate and adapt to increased fire are needed to sustain forest landscapes. Identifying and implementing appropriate responses will not be easy because the complexity of local-to-regional dynamics makes uniform, simple, or unchanging policy and management strategies ineffective (10). It is especially difficult to motivate social response to environmental transitions that unfold slowly and are thus difficult to detect before it is too late (11).

We suggest strategies for forests of all fire regimes: *Landowners should follow “Firewise” guidelines* (www.firewise.org/) *for houses and other infrastructure*. Increased development in fire-prone landscapes has increased suppression costs, exacerbated risk to human safety and infrastructure, and reduced management options. People living in these forests must be prepared rather than relying solely on fire departments. Some places may be so hazardous that building should be prevented, discouraged, or removed (e.g., by regulation or insurance and/or tax incentives).

Fire managers should avoid trying to uniformly blacken wildfire landscapes through burnout and mop-up operations, especially in burn interiors. As wildfire sizes have grown in recent decades, direct attack has been replaced with indirect attack, where fire lines are placed some distance from the active fire front, and then the area between is intentionally burned, often with high-severity fire, to reduce fuel and create a wider fire barrier. Unburned or partially burned patches are critical refugia that aid postfire recovery in forests of all fire regimes and should be conserved whenever possible.

Land managers could anticipate changes using models of species distribution and ecological processes and should consider using assisted migration (12). Dominant forest species may be unable to recover from fires with large high-severity patches. Replacement ecosystems of shrublands or grasslands may provide some ecological benefits, but they offer very different habitats for wildlife and have reduced carbon storage relative to native forests.

We also suggest several distinct strategies based on a forest’s historical fire regime. *Mitigation in forests with historically high-frequency, low- to moderate-severity fire regimes*: (i) Restore resilient forest structure similar to historical patterns that survived during past high-fire periods (and those anticipated in the future) (see the figure). Fuel reduction and restoration treatments can increase resiliency by reducing density-dependent tree mortality (4) and excessive insect and/or disease problems and can increase spatial heterogeneity.

(ii) Fund forest restoration. We know how to treat forests to reduce fire hazards, with generally positive or neutral ecological effects, although impacts to wildlife with large home ranges have not been fully assessed (13). Public acceptance of these treatments is increasing (14); the barrier is cost. Treatment rates are far below what is needed for landscape resilience (15). Because the federal government has no jurisdiction in development policies in the privately owned urban-wildland interface, state and local jurisdictions could pay for fire suppression in the interface. This would enable a significant increase in critical forest restoration funding and would probably reduce building in the interface.

Adaptation in forests with historically low-frequency, high-severity fire regimes: (i) Expect changes in forest type and age across the landscape (see the figure). Some forest types will be relatively resilient to more frequent fires, notably resprouting or seed-banking species. However, even these

forests will likely exhibit substantial changes in landscape structure, such as shifts to a preponderance of young stands (16).

(ii) Some forests will change to nonforest vegetation after fire. Spruce-fir (*Picea-Abies*) and interior Douglas fir (*Pseudotsuga menziesii*) forests may exhibit large changes in structure and species composition because they lack persistent seed banks or sprouting capability. Some areas may even shift to a nonforest state, especially if trees cannot reestablish in a warmer, drier climate. Such changes will not necessarily be catastrophic (e.g., a shift to nonforest could potentially increase water yield) and could be expected to reduce intensity of subsequent fires. However, shifting from forest to nonforest would affect most ecosystem services. There are no clear guidelines for increasing the resilience of these forest types—unlike for forests adapted to high-frequency, low- to moderate-severity fire regimes—other than minimizing additional stresses from excessive grazing, recreation, and salvage logging.

The annual cost of fire suppression is increasing and unsustainable; costs exceeded \$2 billion in the United States in 2012. Fire policy that focuses on suppression only delays the inevitable, promising more dangerous and destructive future forest fires. In contrast, land management agencies could identify large fire sheds (20,000 to 50,000 ha) where, under specified weather conditions, managed wildfire and large prescribed fire are allowed to burn, sometimes after strategic mechanical fuel treatments (15). Acknowledging diversity in fire ecology among forest types and preparing forests and people for larger and more frequent fires could help reduce detrimental consequences.

References

1. T. Schoennagel et al., *BioSci.* **54**, 661 (2004).
2. J. K. Balch et al., *Glob. Change Biol.* **19**, 173–183 (2013).
3. P. Attiwill, D. Binkley, *For. Ecol. Manage.* **294**, 1–3 (2013).
4. A. P. Williams et al., *Nat. Clim. Change* **3**, 292–297 (2013).
5. M. A. Moritz et al., *Ecosphere* **3**, art49 (2012).
6. J. S. Littell et al., *Ecol. Apps.* **19**, 1003–1021 (2009).
7. M. Moriondo et al., *Clim. Res.* **31**, 85–95 (2006).
8. M. Flannigan et al., *Glob. Change Biol.* **15**, 549–560 (2009).
9. A. L. Westerling et al., *Science* **313**, 940–943 (2006).
10. F. S. Chapin et al., *BioSci.* **58**, 531 (2008).
11. T. P. Hughes et al., *Trends Ecol. Evol.* **28**, 149–155 (2013).
12. L. R. Iverson, D. McKenzie, *Landscape Ecol.* **28**, 879–889 (2013).
13. S. L. Stephens et al., *BioSci.* **62**, 549–560 (2012).
14. S. M. McCaffrey, C. C. Olsen, “Research perspectives on the public and fire management: A synthesis of current social science on eight essential questions” (Gen. Tech. Rep. NRS-104, U.S. Department of Agriculture Forest Service, Newtown Square, PA, 2012).
15. M. P. North et al., *J. For.* **110**, 392–401 (2012).
16. A. L. Westerling et al., *Proc. Natl. Acad. Sci. U.S.A.* **108**, 13165–13170 (2011).

10.1126/science.1240294



Future Science

James A. Evans

Science **342**, 44 (2013);

DOI: 10.1126/science.1245218

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/44.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/44.full.html#related>

This article **cites 11 articles**, 6 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/44.full.html#ref-list-1>

Future Science

James A. Evans

The massive growth of global research activity in recent years has spurred studies exploring how productive this expansion has been and what the future may hold. Although the creativity and serendipity of individual discoveries will remain difficult to model, quantitative research has revealed regularities in the rates of discovery and the outcome of published findings over time (1). Some of these studies demonstrate that innovation has been decreasing, which may reflect the output of a scientific enterprise whose system of input has supported the pursuit of research focused on “low-hanging fruit” (2). Coincident with this decline has been the recent global recession, a circumstance that called for accountability of economic and social returns from public investments in research (3). There is now a great demand for insight into how the system of science works. On page 127 of this issue, Wang *et al.* (4) offer one approach to assess, and perhaps even augment, scientific productivity.

An emerging area of interest in research on the “science of science” is the prediction of future impact. Impact prediction is consequential for the evaluation of research grants, the dispensing of scholarly awards, and the determination of faculty salaries, among other decisions. As predictions improve, they will play a larger role in directing choices about what areas public and private capital will choose to research, develop, and produce. But how can we predict the future?

A number of recent studies have honed our understanding of the factors that influence future citations to an article or researcher. For example, citations accrue to articles published in scientific journals over time according to a well-behaved log-normal distribution, with a rise in citations at the point of publication followed by a gradual decay (5). There is also a strong “first-mover” advantage to the receipt of citations—that is, an early mediocre article on a topic will often receive more citations than a later excellent one (6). There are still other factors that predict citations to a scientist’s complete oeuvre, such as the subject diversity of journals in which the scientist’s early articles were published (7). Wang *et al.* advances these efforts by integrating



Winners foreseen. Methods to predict future citations of an article could improve the overall productivity of the scientific enterprise.

three key features into a generative model of an article’s long-term impact: preferential attachment, a temporal citation trend, and the underlying “fitness” of the paper. Their model links previous models of what accounts for the popularity of Web sites [the preferential attachment with which other Web sites hyperlink to it (8) and the “quality” of the Web sites’s content, reflected in its utility for Internet users (9)] and the log-normal probability with which an article’s influence rises then falls over time. The authors combined these elements into a three-parameter model with a number of clear, derivable, and empirically estimable quantities associated with an individual article. These include the article’s relative fitness (its importance relative to its peers), immediacy (the time required to reach its citation peak), longevity (its rate of citation decay), impact time (the characteristic time to attract the bulk of its citations), and ultimate impact (the citations it will acquire over its lifetime, which depends only on its relative fitness). The resulting model was used to predict later citations on the basis of early patterns. By accounting for these different features, all nonzero citation trajectories followed a similar path. Moreover, the authors generalize the model to the level of journals (as they could for researchers, departments, universities, countries, or the scientific system as a whole) to explain how a recent drop in the relative impact of a well-known journal can be solely accounted for by the rising impact time associated with its articles.

We should consume these explanations and the concepts underlying them with cau-

Can predicting an article’s success change science?

tion (as we should with any simplifying model). For example, “fitness,” which is well-named, is not merely quality—the “inherent” or timeless value of an article to science—but the fit between an article and its perceived importance to an audience at the time of publication. Fitness is neither “inherent” nor timeless. With relevant developments in other areas of science, an article’s ideas may become fit. The model of Wang *et al.* is also not magical; its estimation of the

future improves with more past data. Moreover, citations represent only a single dimension of impact, because nonacademic practitioners may be influenced by an article but cannot cite it. Nonetheless, the model assembles a number of important factors for predicting future citation success.

The success of this model and others that will build on it raises the question of how predicting an article’s success could change science. Widespread, consistent prediction of an idea’s future impact will necessarily speed the resolution of which ideas win and which lose in the competition for attention and resources. Because we cannot know everything about the future, knowing only the momentum of an article’s reception could act as a self-fulfilling prophecy—thus, scientists and funders could prematurely abort ideas that may yet have a second or third act to play. The classic work by mathematicians Erdős and Rényi on random networks, which much later became the foundation for popular approaches in network science, is such an example; Wang *et al.* admit that their model could not have predicted future citations of this seminal work. The ability to better predict an article’s success could translate into a faster scientific life cycle for the discovery—from time of publication to widespread acceptance. This might then translate into faster convergence to best practices that would boost the number of scientists with skills required to build on an impactful discovery. This could happen, however, only because rapid swarming around new ideas will increase competition for follow-on research and publication, as

Department of Sociology, University of Chicago, Chicago, IL 60637, USA. E-mail: jevans@uchicago.edu

a larger proportion of scientists pursue the same anointed path.

These cautions, however, do not apply to all that more successful impact prediction portends. The ability to automatically extract scientific claims from research articles and reason across them should lead to the prediction or computational generation of promising new hypotheses. It likely will also expose common assumptions and practices of science to scrutiny and explicit evaluation (10). In this way, citation prediction represents one step on the path to creating algorithmic or

robot “scientists” (11) that are more creative, risky, persistent, and wide-reading than ourselves (12). By enabling scientists to consider not only the most fruitful hypothesis but also the most fruitful algorithm for generating hypotheses, future prediction methods would augment scientific ability, increase productivity, and multiply returns from science for society.

References

1. S. Arbesman, *The Half-Life of Facts: Why Everything We Know Has an Expiration Date* (Current, New York, 2012).
2. B. F. Jones, *Rev. Econ. Stud.* **76**, 283 (2009).

3. J. Lane, *Science* **324**, 1273 (2009).
4. D. Wang, C. Song, A.-L. Barabási, *Science* **342**, 127 (2013).
5. M. J. Stringer, M. Sales-Pardo, L. A. Nunes Amaral, *PLoS ONE* **3**, e1683 (2008).
6. M. E. J. Newman, *Europhys. Lett.* **86**, 68001 (2009).
7. D. E. Acuna, S. Allesina, K. P. Körding, *Nature* **489**, 201 (2012).
8. A.-L. Barabási, R. Albert, *Science* **286**, 509 (1999).
9. G. Bianconi, A.-L. Barabási, *Europhys. Lett.* **54**, 436 (2001).
10. J. A. Evans, J. G. Foster, *Science* **331**, 721 (2011).
11. R. D. King *et al.*, *Science* **324**, 85 (2009).
12. J. A. Evans, A. Rzhetsky, *Science* **329**, 399 (2010).

10.1126/science.1245218

PLANT SCIENCE

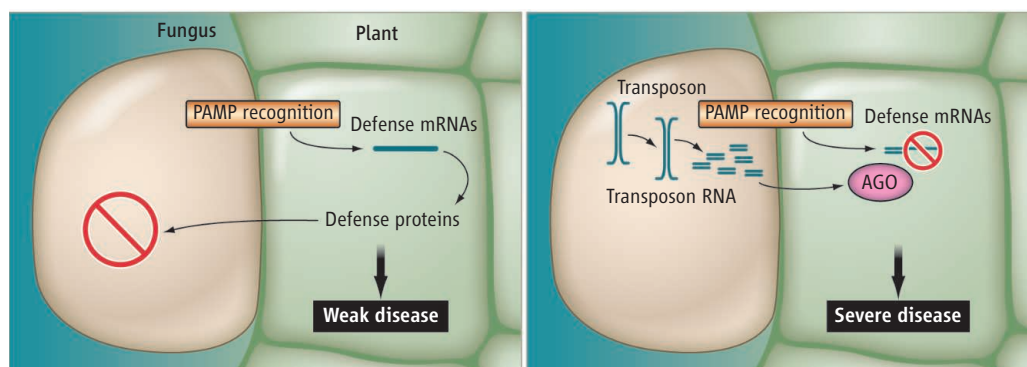
Small RNA—the Secret of Noble Rot

David Baulcombe

Benjamin Franklin should have included infectious disease as a third certainty of life alongside death and taxes. We will always be plagued by pests and pathogens because they generally have shorter life cycles than their hosts, allowing them to evolve rapidly to avoid host defenses. The use of antibiotics, for example, may select for drug-resistant strains of bacteria and, in agriculture, the widespread cultivation of a disease-resistant crop is often followed by the appearance of resistance-breaking strains of the pathogen.

Whenever a host acquires a novel defense, the pest will, eventually, evolve a corresponding counter-defense system. On page 118 of this issue, Weiberg *et al.* (1) describe a strategy in which a fungal parasite uses RNA to block a host's defense system

In plants, the evolutionary arms race involves a set of host receptors that recognize components on the surface of the pathogen (2). These pathogen-associated molecular patterns (PAMPs) include, for example, flagellar proteins of Gram-negative bacteria and chitin in the cell wall of fungi. Triggering these receptors leads to intracellular signaling within and biochemical changes in and around the infected cell such that the invad-



Fungal sRNAs affect disease progression. In the absence of fungal sRNA (left) the pathogen-associated molecular pattern (PAMP) recognition system ensures that the fungus is suppressed and disease is weak. By contrast, in their presence (right), the host defense system is blocked, and the disease progresses rapidly.

ing pathogen is suppressed. Counter-defense then involves pathogen-derived effectors that either block the initial recognition on the surface of the host cell, or are transferred into the infected cell where they suppress the signaling pathways or biochemical changes. However, the arms race does not end there. The plant has a second set of receptors that recognize the effectors and trigger additional layers of defense. The pathogen, correspondingly, has additional counter-defense systems (2).

Most of the characterized pathogen effectors are proteins, but Weiberg *et al.* (1) demonstrate that we can add RNA to the list of effectors with trans-kingdom activity. The new research involves *Botrytis cinerea*—a necrotrophic fungal pathogen that infects many plant species, including tomato and strawberry, on which it causes gray mold.

Small RNA from a fungal pathogen is transferred to cells of a plant host where it silences the mRNA for defense proteins.

Most notably, it is the noble rot that is so important for the production of exquisite dessert wines.

The new findings involve a class of small RNAs (sRNAs) that includes microRNAs and small interfering RNAs. These sRNAs are typically 20 to 24 nucleotides in length and they guide Argonaute (AGO) nucleases by Watson-Crick base pairing to coding or noncoding RNAs in either the nucleus or cytoplasm so that the targeted RNAs accumulate at a lower level than they would in the absence of the sRNA (3).

There are more than 800 fungal sRNAs that are induced when *Botrytis* is infecting plant cells and, of these, 73 were predicted from their sequence to silence host mRNAs, including some that have a role in immunity (1). This silencing potential could be due to

Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK. E-mail: dcb40@cam.ac.uk



Small RNA—the Secret of Noble Rot

David Baulcombe

Science **342**, 45 (2013);

DOI: 10.1126/science.1245010

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/45.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/45.full.html#related>

This article **cites 6 articles**, 3 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/45.full.html#ref-list-1>

a larger proportion of scientists pursue the same anointed path.

These cautions, however, do not apply to all that more successful impact prediction portends. The ability to automatically extract scientific claims from research articles and reason across them should lead to the prediction or computational generation of promising new hypotheses. It likely will also expose common assumptions and practices of science to scrutiny and explicit evaluation (10). In this way, citation prediction represents one step on the path to creating algorithmic or

robot “scientists” (11) that are more creative, risky, persistent, and wide-reading than ourselves (12). By enabling scientists to consider not only the most fruitful hypothesis but also the most fruitful algorithm for generating hypotheses, future prediction methods would augment scientific ability, increase productivity, and multiply returns from science for society.

References

1. S. Arbesman, *The Half-Life of Facts: Why Everything We Know Has an Expiration Date* (Current, New York, 2012).
2. B. F. Jones, *Rev. Econ. Stud.* **76**, 283 (2009).

3. J. Lane, *Science* **324**, 1273 (2009).
4. D. Wang, C. Song, A.-L. Barabási, *Science* **342**, 127 (2013).
5. M. J. Stringer, M. Sales-Pardo, L. A. Nunes Amaral, *PLoS ONE* **3**, e1683 (2008).
6. M. E. J. Newman, *Europhys. Lett.* **86**, 68001 (2009).
7. D. E. Acuna, S. Allesina, K. P. Körding, *Nature* **489**, 201 (2012).
8. A.-L. Barabási, R. Albert, *Science* **286**, 509 (1999).
9. G. Bianconi, A.-L. Barabási, *Europhys. Lett.* **54**, 436 (2001).
10. J. A. Evans, J. G. Foster, *Science* **331**, 721 (2011).
11. R. D. King *et al.*, *Science* **324**, 85 (2009).
12. J. A. Evans, A. Rzhetsky, *Science* **329**, 399 (2010).

10.1126/science.1245218

PLANT SCIENCE

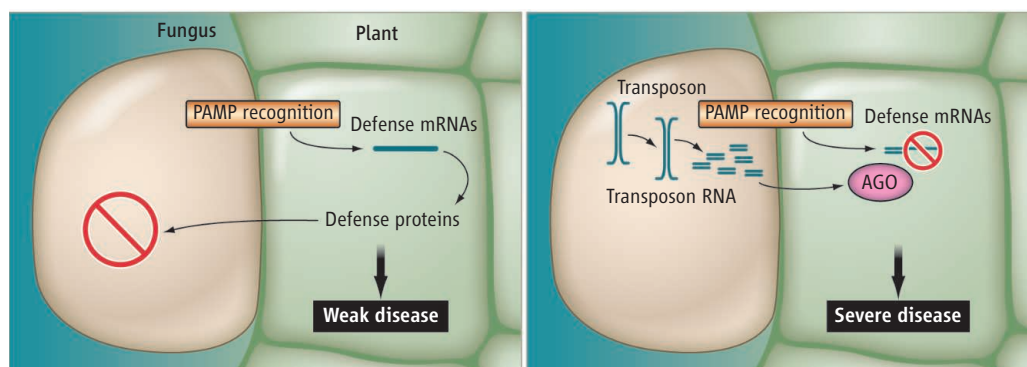
Small RNA—the Secret of Noble Rot

David Baulcombe

Benjamin Franklin should have included infectious disease as a third certainty of life alongside death and taxes. We will always be plagued by pests and pathogens because they generally have shorter life cycles than their hosts, allowing them to evolve rapidly to avoid host defenses. The use of antibiotics, for example, may select for drug-resistant strains of bacteria and, in agriculture, the widespread cultivation of a disease-resistant crop is often followed by the appearance of resistance-breaking strains of the pathogen.

Whenever a host acquires a novel defense, the pest will, eventually, evolve a corresponding counter-defense system. On page 118 of this issue, Weiberg *et al.* (1) describe a strategy in which a fungal parasite uses RNA to block a host's defense system

In plants, the evolutionary arms race involves a set of host receptors that recognize components on the surface of the pathogen (2). These pathogen-associated molecular patterns (PAMPs) include, for example, flagellar proteins of Gram-negative bacteria and chitin in the cell wall of fungi. Triggering these receptors leads to intracellular signaling within and biochemical changes in and around the infected cell such that the invad-



Fungal sRNAs affect disease progression. In the absence of fungal sRNA (left) the pathogen-associated molecular pattern (PAMP) recognition system ensures that the fungus is suppressed and disease is weak. By contrast, in their presence (right), the host defense system is blocked, and the disease progresses rapidly.

ing pathogen is suppressed. Counter-defense then involves pathogen-derived effectors that either block the initial recognition on the surface of the host cell, or are transferred into the infected cell where they suppress the signaling pathways or biochemical changes. However, the arms race does not end there. The plant has a second set of receptors that recognize the effectors and trigger additional layers of defense. The pathogen, correspondingly, has additional counter-defense systems (2).

Most of the characterized pathogen effectors are proteins, but Weiberg *et al.* (1) demonstrate that we can add RNA to the list of effectors with trans-kingdom activity. The new research involves *Botrytis cinerea*—a necrotrophic fungal pathogen that infects many plant species, including tomato and strawberry, on which it causes gray mold.

Small RNA from a fungal pathogen is transferred to cells of a plant host where it silences the mRNA for defense proteins.

Most notably, it is the noble rot that is so important for the production of exquisite dessert wines.

The new findings involve a class of small RNAs (sRNAs) that includes microRNAs and small interfering RNAs. These sRNAs are typically 20 to 24 nucleotides in length and they guide Argonaute (AGO) nucleases by Watson-Crick base pairing to coding or noncoding RNAs in either the nucleus or cytoplasm so that the targeted RNAs accumulate at a lower level than they would in the absence of the sRNA (3).

There are more than 800 fungal sRNAs that are induced when *Botrytis* is infecting plant cells and, of these, 73 were predicted from their sequence to silence host mRNAs, including some that have a role in immunity (1). This silencing potential could be due to

Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, UK. E-mail: dcb40@cam.ac.uk

chance similarity of sequence motifs in the fungal and host-plant genomes and therefore have no functional importance. However, expression of the putative target genes was reduced in *Botrytis*-infected plants and, if their expression was blocked experimentally, the plants became hypersusceptible to the fungus (1). It is likely, therefore, that fungal sRNA silences the expression of host defense genes so that the plant cell is less able to resist the fungus attack.

Further support for this idea comes from the findings that fungal sRNAs bind to a plant AGO in the infected cells, and from the reduced fungal susceptibility of *AGO* mutant plants. The fungal sRNA is likely to be processed from a long RNA precursor into an sRNA before it is translocated into the plant cell (see the figure) (1) because knockout of the fungal Dicer enzyme responsible for sRNA biogenesis also led to reduced fungal susceptibility. Reduced amounts of the plant Dicer, by contrast, had no effect.

Of the 73 *Botrytis* sRNAs with the potential to silence plant genes in the infected plant cell, 52 were derived from mobile DNA elements of the fungal genome. This finding is not surprising—RNA silencing is well established as a defense system and many organisms use sRNAs to silence parasitic trans-

posable elements and thereby protect their genomes (even parasites have parasites). However, the association of transposon sRNA with the suppression of host defenses may have important implications for the host pathogen arms race. Transposable elements are normally the most labile features in eukaryotic genomes (4). Perhaps a DNA transposition event triggers novel patterns of sRNA in the fungus of which, by chance, some can silence additional host defense genes and thereby increase the virulence or fitness of the pathogen. Such a system would further enhance the adaptation of a short-life cycle pathogen to the host defense systems.

The finding of Weiberg *et al.* with *Botrytis* is not the first report of RNA traffic between plants and their pathogenic fungi. Other recent work reveals how RNAs also move from plants into fungal cells, where they silence fungal genes (5). Until now, this “host-induced gene silencing” has been used to silence different fungal genes to determine whether they affect the disease-causing ability of the fungus. It remains to be tested whether endogenous plant sRNAs are also moved into the fungus. However, given the abundance of sRNAs in plant cells, it would be surprising if they did not both enter the fungus and silence complementary RNA.

There is clearly much more to be discovered about RNA-based communication between plants and pathogenic fungi. More information is needed about the transport mechanism and whether it applies to biotrophic pathogens (5) (which form a long-term feeding relationship with their host), as well as necrotrophic pathogens. Another question is whether this transfer of RNA between plants and fungi is related to other “social RNA” in animals that may affect interactions between cells and organisms (6). In addition, the possibility should be explored that another layer exists in the arms race in which the plant blocks the effects of the transferred RNA, and whether the information can be exploited to develop management or control strategies for plant disease. It would be very pleasing if the answers to these questions lead to Chateau d'Yquem in the price range of a graduate student.

References

1. A. Weiberg *et al.*, *Science* **342**, 118 (2013).
2. J. D. Jones, J. L. Dangl, *Nature* **444**, 323 (2006).
3. D. Baulcombe, *Nature* **431**, 356 (2004).
4. D. Lisch, *Nat. Rev. Genet.* **14**, 49 (2013).
5. D. Nowara *et al.*, *Plant Cell* **22**, 3130 (2010).
6. P. Sarkies, E. A. Miska, *Science* **341**, 467 (2013).

10.1126/science.1245010

CHEMISTRY

Turn the Molecule This Way for a Faster Reaction

Michael C. Heaven

Observed under normal conditions, chemical reaction rates and the relative ratios of products formed are highly averaged properties. These values sum over elementary events that sample wide ranges of collision energy, internal energies of the reactants, relative orientations, and collision strengths (e.g., glancing to head-on). It has been a long-standing challenge to look inside this statistical averaging to discern the intimate details of reaction dynamics (1). Many techniques have been developed that permit partial selection and control of the physical states of reactants before their encounters. As the averaging is stripped away, a deeper understanding of chemical reaction

mechanisms begins to emerge. On page 98 of this issue, Chang *et al.* (2) describe a new approach to studies of bimolecular reactions using conformer-selected molecules and a target of ultracold atoms.

To demonstrate the capabilities of their method, Chang *et al.* have shown that two similar conformers of 3-aminophenol (*cis*- and *trans*-3AP, see the figure) have substantially different cross sections—the probability that they will react—with singly charged calcium ions (Ca^+). Three levels of control were exercised in these experiments. First, a beam of 3AP molecules was formed by expanding the vapor (mixed with a high pressure of inert neon buffer gas) into a vacuum. This process cooled the vibrational and rotational motions of the molecules to a temperature near 1 K, which prevented interconver-

Molecular beams and ultracold atom-trapping methods resolve the different reaction rates of the *cis* and *trans* conformers of a flexible organic molecule.

sion between the conformers and compressed the velocity distribution to a narrow range. Hence, for this reagent, the translational and internal energies were sharply defined.

Spatial separation of the conformers, the second level of control, was accomplished by having the molecular beam traverse a region where a strong electric field bent the trajectories (3). The field exerted a force on the molecules through interaction with the molecular electric dipole moment. A key feature of this experiment is that the two conformers have markedly different dipole moments (4). The *cis* conformer, which has the larger dipole, deflected more.

Manipulation of molecules with molecular beams and field deflection is challenging, but the techniques are established. It is the third level of control applied in the experi-

Department of Chemistry, Emory University, Atlanta, GA 30322, USA. E-mail: heaven@euch4e.chem.emory.edu



Social Factors in Epidemiology

Chris T. Bauch and Alison P. Galvani

Science **342**, 47 (2013);

DOI: 10.1126/science.1244492

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

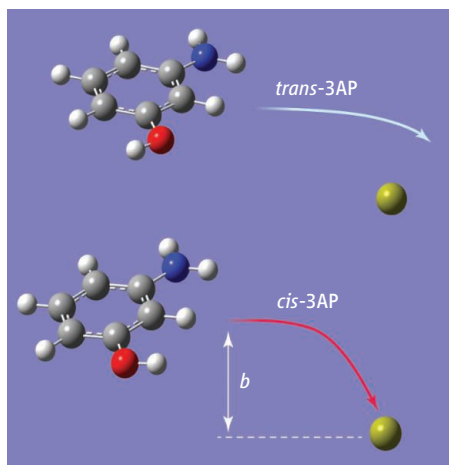
<http://www.sciencemag.org/content/342/6154/47.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/47.full.html#related>

This article **cites 12 articles**, 3 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/47.full.html#ref-list-1>



ments of Chang *et al.* that required new methods. To achieve the desired specification of the reactive encounters, the Ca^+ ions must have well-defined velocities and be spatially confined. These conditions were met by using electro-optical cooling and trapping. A Coulomb crystal of ultracold Ca^+ ions was created at the center of an ion trap. In this construct, the ions were arranged in an ordered lattice held in place by external fields (5). The lasers that cooled the ions also generate atomic fluorescence that was used to obtain an image of the crystal. After the trap was loaded, reactive loss of the ions was detected as a shrinking of the crystal image. The individual ions were separated by distances on the order of 20 to 30 μm and acted as isolated atoms during a reactive encounter.

Chang *et al.* found that *cis*-3AP reacted with Ca^+ with a cross section that was ~ 1.5 times the size of that for *trans*-3AP. Like many ion-molecule reactions, $\text{Ca}^+ + 3\text{AP} \rightarrow \text{products}$ has no energetic barrier. Any encounter that brings the collision pair closer than a critical distance results in reaction. Thus, the long-range attractive forces acting between the collision pair play an important role in determining the reaction cross section (6). The dynamics can be imagined in terms of a capture model. If the 3AP molecule comes close enough to the Ca^+ ion, it will be pulled inside the critical distance and reaction will occur. The 3AP trajectory is deflected if the approach is not within this radius, but there is no reaction (as illustrated in the figure for *trans*-3AP).

The long-range attraction between Ca^+ and 3AP is dominated by the charge-dipole interaction and polarization (dispersion) effects. The latter are not much influenced by *cis-trans* isomerization, but the dipole moment changes dramatically (4). Roughly speaking, the dipole can be described as the vector sum of the dipoles of the HOC and $\text{C}_6\text{H}_4\text{NH}_2$ fragments. These sums are reinforced or partially

More attractive is more reactive. The *trans* and *cis* conformers of 3AP are shown (carbon atoms are in gray, hydrogen in white, oxygen in red, nitrogen in blue, and calcium in gold). In the experiment of Chang *et al.*, these molecules are directed at the Ca^+ ions with the same initial velocity ($\sim 910 \text{ m s}^{-1}$). Trajectories are indicated for an initial impact displacement (b) of 9.5 Å. For this choice, the *cis* conformer is captured, but the less attractive *trans* conformer flies on by.

cancelling in the *cis* and *trans* conformers, respectively. It is the greater dipole moment of the *cis* conformer that yields the larger attractive force and reaction cross section. As noted above, this difference in the dipoles is also the discriminating factor used for the spatial separation of the 3AP conformers.

In establishing a new technique, it is essential to begin with examples where the outcome can be reasonably well anticipated. This elegant demonstration experiment identifies the single overriding principle that controls the $\text{Ca}^+ + 3\text{AP}$ reaction. Chang *et al.* have shown that ions held in ultracold Coulomb crystals can be used to investigate the complex dynamics of the reactions of atoms with polyatomic molecules. The control of the molecules in this demonstration was achieved by expansion cooling and conformer separation. In future experiments,

electric and magnetic field may be tailored to control the orientation of the molecules (7, 8), and laser excitation can be used to modulate the internal energy. This merging of molecular beam technology with the physics of laser cooling and ion trapping adds a valuable new tool for investigations of reaction mechanisms at the most fundamental level.

References

1. P. L. Houston, *Chemical Kinetics and Reaction Dynamics* (McGraw-Hill, New York, 2001).
2. Y.-P. Chang *et al.*, *Science* **342**, 98 (2013).
3. F. Filsinger *et al.*, *Angew. Chem. Int. Ed.* **48**, 6900 (2009).
4. F. Filsinger, K. Wohlfart, M. Schnell, J.-U. Grabow, J. Küpper, *Phys. Chem. Chem. Phys.* **10**, 666 (2008).
5. S. Willitsch, M. T. Bell, A. D. Gingell, T. P. Softley, *Phys. Chem. Chem. Phys.* **10**, 7200 (2008).
6. J. Troe, *J. Chem. Phys.* **87**, 2773 (1987).
7. F. Filsinger *et al.*, *J. Chem. Phys.* **131**, 064309/1 (2009).
8. S. Y. T. van de Meerakker *et al.*, *Nat. Phys.* **4**, 595 (2008).

10.1126/science.1244833

EPIDEMIOLOGY

Social Factors in Epidemiology

Chris T. Bauch¹ and Alison P. Galvani^{1,2}

The coupling of social and biological contagion in human populations can have positive or negative outcomes.

Despite the invention of control measures like vaccines, infectious diseases remain part of human existence. Ideas, sentiments, or information can also be contagious (1, 2). Such social contagion is akin to biological contagion: Both spread through a replication process that is blind to the consequences for the individual or population, and if each person transmits to more than one person, the explosive power of exponential growth creates an epidemic. Social contagions may cause irrational “fever.” Isaac Newton, having lost £20,000 in the speculative South Sea Bubble, commented that he could “calculate the movement of the stars, but not the madness of men” (3). Systems in which both contagion types are coupled to one another—an infectious disease spreading by biological

contagion and a social contagion concerning the disease—offer unique scientific challenges and are increasingly important for public health (4–15).

Social contagions can exhibit complex dynamics, such as the tipping points and cascading instabilities observed in financial collapse (2). Social contagion can be conceptualized as a network, where each node is a person, and the network links are social contacts through which the contagion can spread (see the figure, panel A). Infectious diseases can also exhibit complex dynamics and be conceptualized as a network through which the biological contagion spreads (see the figure, panel B). When a social contagion is coupled to a biological contagion, the resulting disease-behavior system can exhibit dynamics that do not occur when the two subsystems are isolated from one another. This illustrates the lesson of complexity science that the whole is more than the sum of the parts (see the figure, panel C).

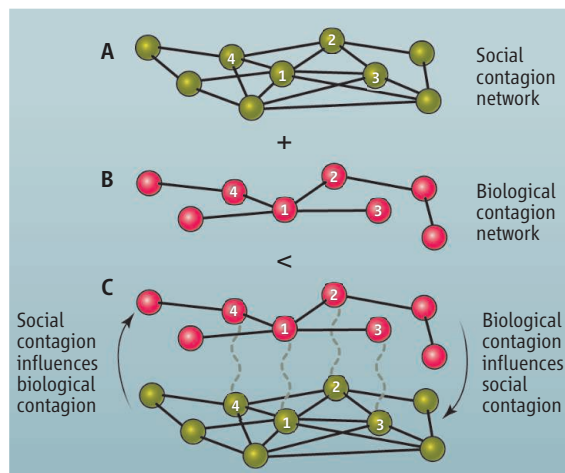
¹Department of Applied Mathematics, University of Waterloo, Waterloo, ON N2L 3G1, Canada ²School of Public Health, Yale University, New Haven, CT 06520, USA. E-mail: alison.galvani@yale.edu

For example, high levels of pediatric vaccine coverage can decrease disease incidence to very low levels, reducing the perceived danger of infection and hence the urgency to get vaccinated. Subsequently, if highly connected nodes in the social network (such as celebrities) suggest that the vaccine carries risks, the resulting perception of vaccine risks can propagate quickly through the social network, fueling a vaccine scare and a drop in vaccine coverage. In this case, biological contagion influences social contagion (see the figure, panel C).

In turn, the drop in vaccine coverage allows the number of individuals who are susceptible to infection to accumulate. When the percentage of susceptible individuals exceeds a tipping point, an outbreak of infectious disease occurs, which may motivate individuals to once again seek vaccination: Social contagion influences biological contagion (see the figure, panel C). This dynamic may have occurred during whole-cell pertussis vaccine scare in the United Kingdom during the 1970s and the more recent measles-mumps-rubella vaccine autism scare (4). Similarly, in some populations, the advent of antiviral HIV drugs led to a rise in risky sexual behavior, and consequently a rise in sexually transmitted infections (5).

However, social contagions can also produce positive consequences in disease-behavior systems. Social norms dictate that individuals should cover their mouth when sneezing. Parents often vaccinate their children because other parents around them do so. Altruism can also be an important motivator (6). The Israeli Ministry of Health recently called for a dose of oral polio vaccine (OPV) to be given to children who had previously been immunized with inactivated polio vaccine (IPV). The primary goal was to prevent infection in those with weakened immune systems who cannot get vaccinated; in contrast to IPV, OPV prevents viral shedding and thus protects individuals in contact with the vaccinated person. Consequently, in this case vaccination with OPV was largely an act of altruism. Polio vaccine uptake exceeded the initial targets within 8 days of the call by the Israeli Ministry of Health (7).

Public health communications regarding the dangers of infection are prevalent for myriad infectious diseases. In many cases,



More than the sum of its parts. When a social contagion on a social network (A) and a biological contagion on an infection contact network (B) are coupled to one another (C), the resulting interplay can create complex dynamics that are not present in either network layer in isolation, such as the fall and rise of vaccine coverage during a vaccine scare (4). Each person is a node in both the social and the biological contagion network.

these public health communications have a beneficial effect on behavior. In other cases, their message is eclipsed by the influences of peers in social networks and by direct personal experience with infection or vaccination. The complexities of disease-behavior dynamics contribute to this undermining of public health efforts. The feedback loop (see the figure, panel C) results in the readjustment of disease-behavior systems following perturbations, such as public health efforts to change behavior.

Epidemic trajectories and the uptake of control measures can vary widely between populations. SARS-coronavirus caused large epidemics in some populations but almost no transmission in others (8). Social differences between populations may be one reason for this. Control of SARS-coronavirus depended partly on population acceptance of quarantine and isolation, which is often determined by social norms. The role of disease-behavior interactions in outbreak heterogeneity has received little attention because of the difficulty of quantifying social feedbacks, but exploiting new sources of data such as online social media may help to address this (9).

The challenges of studying disease-behavior systems are generating interesting science. Mathematical modelers are exploring disease-behavior interactions not only in the context of vaccines but also for the emergence of antibiotic resistance and antiviral influenza drug resistance, as well as for social distancing, where people avoid contact with infected individuals (10). Modelers

are exploring how disease-behavior dynamics, such as population susceptibility to vaccine risk, vary with socioeconomic factors (11). Research on behavior-disease interactions in the context of HIV vaccines and antiviral drugs is also returning after a spike of interest in the 1990s.

Mathematical modelers are constructing realistic models tailored to specific systems and public health research questions and testing the empirical validity of those models (4, 6, 12). As part of this trend, increasing amounts of data on social networks are being collected to help formulate and test network-based disease-behavior models (9, 13). Moreover, modelers are incorporating the insights of economists, sociologists, and psychologists into disease-behavior models (4, 6, 10–13).

Empirically validated disease-behavior models could be used, for example, as predictive tools to explore optimal strategies for public health intervention in response to a vaccine scare. Predicting “the madness of men” in disease-behavior systems may appear daunting. However, if social contagions have clear similarities to well-understood biological contagions that can be captured in models, and if the focus is on predicting aggregate population behavior rather than individual actions, predictive models may be feasible. Even an approximate ability to anticipate how populations will respond to new disease control measures could be helpful.

Some predictability seems to exist in these systems. For example, some models predicted that cervical cancer vaccine uptake would be below recommended levels in the United States, which is what eventually occurred (12, 15). Other models have retrospectively predicted vaccine coverage and endemic disease dynamics for historical pediatric vaccine scares (4). Empirical validation of models could be facilitated by more long-term data on the determinants of control measure uptake in the specific context of disease-behavior systems. Also, the field would benefit from a common lexicon. For example, social contagion is often referred to differently even when the underlying mathematical formulation is identical. This can create confusion.

Vaccines and drugs for many long-established infectious diseases are becoming more widely available. Thus, population behavior may replace accessibility as the most challenging barrier to higher uptake of control measures. On the other hand, for many novel zoonoses like SARS-coronavirus, there are initially no pharmaceuti-

cal interventions. Hence, public health must rely on quarantine, movement restrictions, and other measures that require cooperative behavior. Also, where there is lack of epidemiological knowledge in the early stages of a new zoonosis, fear and supposition can easily rush in to fill the void. In all cases, an improved understanding of the interplay between social contagions and biological contagions will help to improve disease control efforts.

References

1. N. A. Christakis, J. H. Fowler, *N. Engl. J. Med.* **357**, 370 (2007).
2. R. M. May, S. A. Levin, G. Sugihara, *Nature* **451**, 893 (2008).
3. J. O'Farrell, *An Utterly Impartial History of Britain—or 2000 Years of Upper Class Idiots In Charge* (Doubleday, London, 2007).
4. C. T. Bauch, S. Bhattacharyya, *PLOS Comput. Biol.* **8**, e1002452 (2012).
5. N. Crepaz, T. Hart, G. Marks, *J. Am. Med. Soc.* **292**, 224 (2004).
6. J. Liu, B. F. Kochin, Y. I. Tekle, A. P. Galvani, *J. R. Soc. Interface* **9**, 68 (2011).
7. Israeli Ministry of Health, www.health.gov.il/NewsAndEvents/SpokesmanMessages/Pages/27082013_2.aspx (2013).
8. WHO, Epidemic curves—severe acute respiratory syndrome (SARS); see www.who.int/csr/sars/epicurve/epiindex/en/ (2013).
9. M. Salathé, C. C. Freifeld, S. R. Mekaru, A. F. Tomasulo, J. S. Brownstein, *N. Engl. J. Med.* **369**, 401 (2013).
10. T. C. Reluga, *PLOS Comput. Biol.* **6**, e1000793 (2010).
11. A. d'Onofrio, P. Manfredi, P. Poletti, *J. Theor. Biol.* **273**, 63 (2011).
12. S. Basu, G. B. Chapman, A. P. Galvani, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19018 (2008).
13. S. Funk, E. Gilad, C. Watkins, V. A. A. Jansen, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6872 (2009).
14. D. M. Kahan, *Science* **342**, 53 (2013).
15. W. W. Williams *et al.*, *Morb. Mortal. Wkly. Rep.* **62**, 66 (2013).

Acknowledgments: C.T.B. acknowledges grant support from NSERC (Natural Sciences and Engineering Research Council of Canada), MEDI (Ministry of Economic Development and Innovation), CIHR (Canadian Institutes of Health Research), and CFI (Canadian Foundation for Innovation). A.P.G. acknowledges grant support from NIH U01 GM15627, NSF SES-1227390, MIDAS (Models of Infectious Disease Agent Study) U01 GM087719, and McDonnell Foundation 1220020114. We are grateful to D. Yamin, K. Atkins, D. Durham, A. Hofman, S. Greenhalgh, and M. Anand for helpful comments.

10.1126/science.1244492

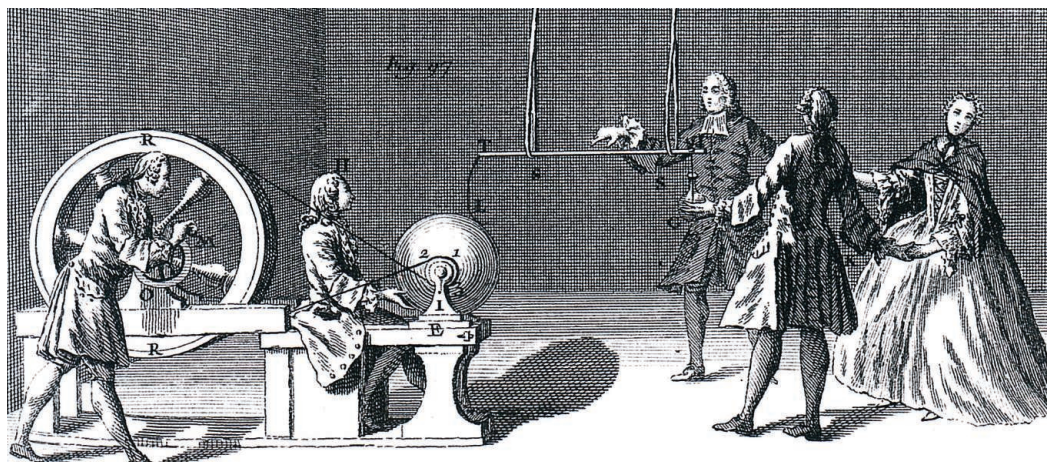
HISTORY OF SCIENCE

Public Science 2.0—Back to the Future

Carsten Könneker^{1,2} and Beatrice Lugger²

There is no denying that the way in which science operates and is communicated both internally and externally is undergoing a fundamental change. Digitization is enabling greater communication and collaboration among experts as well as between experts and lay people, providing opportunities for lay people to become citizen scientists. Yet these upheavals in research and communication processes—referred to by some as Public Science 2.0—are not completely new phenomena. In some ways, science is returning to a relationship with the public that was the norm in earlier times.

In the 18th century, a popular public experimental culture featured “electric theatres,” in which Leyden jars were used for electric stimulations, such as the discharge through an electric kiss or a human chain (see the figure) (1). Panoramas, cosmoramas (precursors of today's planetariums), and cycloramas (panoramic paintings with a 360° view) brought amazing phenomena directly



Electricity en vogue. In the 18th century, people got in touch with the electric thrill in diverse Leyden experiments, here via the human chain (12).

to the people. As early as 1661, Otto von Guericke, amateur scientist and mayor of the German city of Magdeburg, put his findings about “empty space” into the public domain when he showed that 16 horses could not pull apart two brass hemispheres that had had the air pumped out of them.

People have long experimented and gathered observations about the world around them (2), such as collecting data on plant and animal distributions or weather conditions. Toward the end of the 18th century, however, researchers began to conduct their work away from the public eye. Precision measurements with torsion balances, such as those

Modern dialog formats in science communication are reminiscent of a culture of public discourse and involvement in past centuries.

carried out by Charles-Augustin de Coulomb and Henry Cavendish, could be done only in isolated experiment rooms. The advance forced those who had previously performed trials in public to vanish into laboratories. Science presentations in aristocratic circles also diminished when scientists came to be increasingly sponsored by the state rather than aristocratic patrons.

After the withdrawal of researchers from public view, science and its external communication grew apart. Internal scientific communication in respective specialist languages became established. Scientific journals, using peer review, became

¹Faculty of Humanities and Social Sciences, Karlsruhe Institute of Technology, 76128 Karlsruhe, Germany.

²National Institute for Science Communication (NaWik), 76133 Karlsruhe, Germany. E-mail: carsten.koenneker@kit.edu; lugger@nawik.de



Public Science 2.0—Back to the Future

Carsten Könneker and Beatrice Lugger

Science **342**, 49 (2013);

DOI: 10.1126/science.1245848

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/49.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/49.full.html#related>

cal interventions. Hence, public health must rely on quarantine, movement restrictions, and other measures that require cooperative behavior. Also, where there is lack of epidemiological knowledge in the early stages of a new zoonosis, fear and supposition can easily rush in to fill the void. In all cases, an improved understanding of the interplay between social contagions and biological contagions will help to improve disease control efforts.

References

1. N. A. Christakis, J. H. Fowler, *N. Engl. J. Med.* **357**, 370 (2007).
2. R. M. May, S. A. Levin, G. Sugihara, *Nature* **451**, 893 (2008).
3. J. O'Farrell, *An Utterly Impartial History of Britain—or 2000 Years of Upper Class Idiots In Charge* (Doubleday, London, 2007).
4. C. T. Bauch, S. Bhattacharyya, *PLOS Comput. Biol.* **8**, e1002452 (2012).
5. N. Crepaz, T. Hart, G. Marks, *J. Am. Med. Soc.* **292**, 224 (2004).
6. J. Liu, B. F. Kochin, Y. I. Tekle, A. P. Galvani, *J. R. Soc. Interface* **9**, 68 (2011).
7. Israeli Ministry of Health, www.health.gov.il/NewsAndEvents/SpokesmanMessages/Pages/27082013_2.aspx (2013).
8. WHO, Epidemic curves—severe acute respiratory syndrome (SARS); see www.who.int/csr/sars/epicurve/epiindex/en/ (2013).
9. M. Salathé, C. C. Freifeld, S. R. Mekaru, A. F. Tomasulo, J. S. Brownstein, *N. Engl. J. Med.* **369**, 401 (2013).
10. T. C. Reluga, *PLOS Comput. Biol.* **6**, e1000793 (2010).
11. A. d'Onofrio, P. Manfredi, P. Poletti, *J. Theor. Biol.* **273**, 63 (2011).
12. S. Basu, G. B. Chapman, A. P. Galvani, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19018 (2008).
13. S. Funk, E. Gilad, C. Watkins, V. A. A. Jansen, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6872 (2009).
14. D. M. Kahan, *Science* **342**, 53 (2013).
15. W. W. Williams *et al.*, *Morb. Mortal. Wkly. Rep.* **62**, 66 (2013).

Acknowledgments: C.T.B. acknowledges grant support from NSERC (Natural Sciences and Engineering Research Council of Canada), MEDI (Ministry of Economic Development and Innovation), CIHR (Canadian Institutes of Health Research), and CFI (Canadian Foundation for Innovation). A.P.G. acknowledges grant support from NIH U01 GM15627, NSF SES-1227390, MIDAS (Models of Infectious Disease Agent Study) U01 GM087719, and McDonnell Foundation 1220020114. We are grateful to D. Yamin, K. Atkins, D. Durham, A. Hofman, S. Greenhalgh, and M. Anand for helpful comments.

10.1126/science.1244492

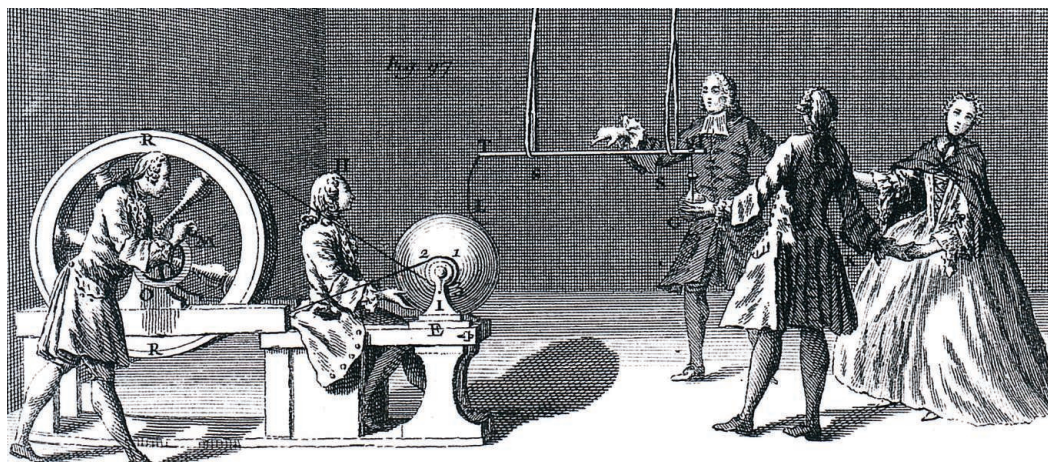
HISTORY OF SCIENCE

Public Science 2.0—Back to the Future

Carsten Könneker^{1,2} and Beatrice Lugger²

There is no denying that the way in which science operates and is communicated both internally and externally is undergoing a fundamental change. Digitization is enabling greater communication and collaboration among experts as well as between experts and lay people, providing opportunities for lay people to become citizen scientists. Yet these upheavals in research and communication processes—referred to by some as Public Science 2.0—are not completely new phenomena. In some ways, science is returning to a relationship with the public that was the norm in earlier times.

In the 18th century, a popular public experimental culture featured “electric theatres,” in which Leyden jars were used for electric stimulations, such as the discharge through an electric kiss or a human chain (see the figure) (1). Panoramas, cosmoramas (precursors of today's planetariums), and cycloramas (panoramic paintings with a 360° view) brought amazing phenomena directly



Electricity en vogue. In the 18th century, people got in touch with the electric thrill in diverse Leyden experiments, here via the human chain (12).

to the people. As early as 1661, Otto von Guericke, amateur scientist and mayor of the German city of Magdeburg, put his findings about “empty space” into the public domain when he showed that 16 horses could not pull apart two brass hemispheres that had had the air pumped out of them.

People have long experimented and gathered observations about the world around them (2), such as collecting data on plant and animal distributions or weather conditions. Toward the end of the 18th century, however, researchers began to conduct their work away from the public eye. Precision measurements with torsion balances, such as those

Modern dialog formats in science communication are reminiscent of a culture of public discourse and involvement in past centuries.

carried out by Charles-Augustin de Coulomb and Henry Cavendish, could be done only in isolated experiment rooms. The advance forced those who had previously performed trials in public to vanish into laboratories. Science presentations in aristocratic circles also diminished when scientists came to be increasingly sponsored by the state rather than aristocratic patrons.

After the withdrawal of researchers from public view, science and its external communication grew apart. Internal scientific communication in respective specialist languages became established. Scientific journals, using peer review, became

¹Faculty of Humanities and Social Sciences, Karlsruhe Institute of Technology, 76128 Karlsruhe, Germany.

²National Institute for Science Communication (NaWik), 76133 Karlsruhe, Germany. E-mail: carsten.koenneker@kit.edu; lugger@nawik.de

the central feature of the scientific process that remained largely hidden from the public. However, some venues for external scientific communication were developed. For example, the Royal Society in Great Britain established the Royal Institution in 1799 to support public engagement with science, mostly through public lectures (3). Such scientists as Albert Einstein considered it part of their duties as researchers to actively communicate results. Einstein gave public lectures (4), published the book *Relativity: The Special and General Theory* in 1917 (5), and even released a vinyl record of him reading out his “credo” in 1932 (6).

An important turning point came with the emergence of modern mass media in the 20th century. It became home to much of the popular and scientific discourse, where journalists and editorial offices selected and tailored the topics according to their own criteria such as news value and relevance, with little room for dialog. “Professional science lost the public of the 18th, 19th and early 20th century to the media,” says sociologist Peter Weingart, summing up the situation at the outset of the 21st century (7).

Today, the internal and external communication processes of science are changing. Researchers can publicly display their work on new media platforms, freeing themselves a little from their dependence on the professional media and interacting more directly with other parts of society. At science slams or the FameLab competition, researchers deliver imaginative presentations, hoping to gain the audience’s vote. In Science Cafés, lay people exchange ideas with experts. Laboratory open days allow the public to take a closer look at research institutions and gain insight into their work. Children’s Universities are aimed specifically at young people. Public and patient forums invite critical dialog with researchers.

Through blogs and blog aggregation sites such as ScienceBlogs, SciLogs, and Scientific American Blogs, researchers engage with each other and the public. Some blogging scientists’ posts reach tens of thousands of users, gaining mass media dimensions; examples include climate debate blogs like RealClimate (8) and KlimaLounge (9). Some researchers are also active on social networks (10). A prominent example is the 2011 Physics Nobel Laureate Brian Schmidt, who tweets as @cosmicpinot. The scientists who are getting involved in social media are moving in the marketplaces of the 21st century.

In these modern forms of science communication, researchers can be addressed as

individuals without a need for a publishing body. By making use of both established and new media for their external communication, scientists are returning to a facet of their profession that was familiar to scientists of former centuries: the translator role. Radical changes are also affecting internal scientific communication and thus the scientific process itself. Scientific publications and data are increasingly openly accessible online. Collaborative platforms enable new forms of literature management, cooperation, and information exchange.

Digital collaboration between scientists often allows nonspecialists to become spectators of the research; in certain projects, they can even participate in the scientific process itself as citizen scientists—for example, by counting stars or snails in projects such as Zooniverse or Evolution MegaLab. With digital options like sharing project ideas and sampling data, citizen science today is potentially available to all (11).

Modern communication brings science a little way back toward where it once came from: the people. Not everyone is happy about this and there are risks associated with Public Science 2.0; for example, sharing concepts, ideas, and data openly on the Internet can result in intellectual property abuse, and excessive engagement in social media may cause a loss of reputation among

peers. However, when practiced skillfully, Public Science 2.0 is likely to inspire laypeople and researchers alike.

References and Notes

1. J. Teichmann, *Vom Bernstein zum Elektron* (3rd ed., Deutsches Museum, Munich, 1998).
2. A. Miller-Rushing, R. Primack, R. Bonney, *Front. Ecol. Environ.* **10**, 285 (2012).
3. www.rigb.org
4. A. Engel, transl., E. Schucking, consultant Ed., *The Collected Papers of Albert Einstein, Volume 7: The Berlin Years: Writings, 1918–1921, English translation supplement* (Princeton Univ. Press, Princeton, NJ, 2002). p.160 and p.261.
5. A. Einstein, *Über die spezielle und die allgemeine Relativitätstheorie (Gemeinverständlich)* (Vieweg, Braunschweig, 1917).
6. A. Einstein, *Verehrte An- und Abwesende. Original audio recordings 1921–1951* (Supposé, Cologne, 2003), CD 1; see also www.einstein-website.de/z_biography/credo.html.
7. P. Weingart, in *Wissenschaft und Hochschulbildung im Kontext von Wirtschaft und Medien*, B. Hölscher, J. Suchanek, Eds. (Springer VS, Wiesbaden, 2011), p. 54. Quote translated by Tom Harris.
8. www.realclimate.org
9. www.scilogs.de/wblogs/blog/kimalounge
10. H. M. Bik, M. C. Goldstein, *PLoS Biol.* **11**, e1001535 (2013).
11. J. Silvertown, *Trends Ecol. Evol.* **24**, 467 (2009).
12. Entladung einer Leidener Flasche über eine Menschenkette, from *Dictionnaire universel de mathématique et de physique*, Paris 1753, volume 1.

Acknowledgments: C.K. founded SciLogs in 2007. B.L. was managing editor of scienceblogs.de when it was launched.

10.1126/science.1245848

PLANT SCIENCE

Fine-Tuning Photosynthesis

Jean-David Rochaix

A potassium channel helps to fine-tune the photosynthetic machinery of plants to changes in environmental conditions.

Most biochemical reactions in living cells are fueled by adenosine triphosphate (ATP) and/or the reducing agent reduced nicotinamide adenine dinucleotide phosphate (NADPH). In plant cells, these components are generated in mitochondria and chloroplasts through the respiratory and photosynthetic electron transfer chains. Nearly 50 years ago, Mitchell (1) proposed a unifying mechanism through which the respiratory and photosynthetic electron transfer reactions are coupled to proton pumping across the inner mito-

chondrial and thylakoid membranes; the thylakoid membranes are located within chloroplasts and contain the protein-pigment complexes that catalyze the primary reactions of photosynthesis (see the figure). This proton movement generates a transmembrane electrochemical gradient called the proton motive force. On page 114 of this issue, Carretero *et al.* (2) show how a two-pore potassium channel helps to regulate the proton motive force.

The proton motive force consists of an electric potential ($\Delta\Psi$) and an osmotic component (ΔpH), both of which are used by the ATP synthase to produce ATP (see the figure) (3). ΔpH also regulates electron transfer between plastoquinol and the cytochrome

Department of Molecular Biology and Department of Plant Biology, University of Geneva, 30 Quai Ernest Ansermet, Geneva 4, 1211 Geneva, Switzerland. E-mail: jean-david.rochaix@unige.ch



Fine-Tuning Photosynthesis

Jean-David Rochaix

Science **342**, 50 (2013);

DOI: 10.1126/science.1244943

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/50.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/50.full.html#related>

This article **cites 10 articles**, 3 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/50.full.html#ref-list-1>

the central feature of the scientific process that remained largely hidden from the public. However, some venues for external scientific communication were developed. For example, the Royal Society in Great Britain established the Royal Institution in 1799 to support public engagement with science, mostly through public lectures (3). Such scientists as Albert Einstein considered it part of their duties as researchers to actively communicate results. Einstein gave public lectures (4), published the book *Relativity: The Special and General Theory* in 1917 (5), and even released a vinyl record of him reading out his “credo” in 1932 (6).

An important turning point came with the emergence of modern mass media in the 20th century. It became home to much of the popular and scientific discourse, where journalists and editorial offices selected and tailored the topics according to their own criteria such as news value and relevance, with little room for dialog. “Professional science lost the public of the 18th, 19th and early 20th century to the media,” says sociologist Peter Weingart, summing up the situation at the outset of the 21st century (7).

Today, the internal and external communication processes of science are changing. Researchers can publicly display their work on new media platforms, freeing themselves a little from their dependence on the professional media and interacting more directly with other parts of society. At science slams or the FameLab competition, researchers deliver imaginative presentations, hoping to gain the audience’s vote. In Science Cafés, lay people exchange ideas with experts. Laboratory open days allow the public to take a closer look at research institutions and gain insight into their work. Children’s Universities are aimed specifically at young people. Public and patient forums invite critical dialog with researchers.

Through blogs and blog aggregation sites such as ScienceBlogs, SciLogs, and Scientific American Blogs, researchers engage with each other and the public. Some blogging scientists’ posts reach tens of thousands of users, gaining mass media dimensions; examples include climate debate blogs like RealClimate (8) and KlimaLounge (9). Some researchers are also active on social networks (10). A prominent example is the 2011 Physics Nobel Laureate Brian Schmidt, who tweets as @cosmicpinot. The scientists who are getting involved in social media are moving in the marketplaces of the 21st century.

In these modern forms of science communication, researchers can be addressed as

individuals without a need for a publishing body. By making use of both established and new media for their external communication, scientists are returning to a facet of their profession that was familiar to scientists of former centuries: the translator role. Radical changes are also affecting internal scientific communication and thus the scientific process itself. Scientific publications and data are increasingly openly accessible online. Collaborative platforms enable new forms of literature management, cooperation, and information exchange.

Digital collaboration between scientists often allows nonspecialists to become spectators of the research; in certain projects, they can even participate in the scientific process itself as citizen scientists—for example, by counting stars or snails in projects such as Zooniverse or Evolution MegaLab. With digital options like sharing project ideas and sampling data, citizen science today is potentially available to all (11).

Modern communication brings science a little way back toward where it once came from: the people. Not everyone is happy about this and there are risks associated with Public Science 2.0; for example, sharing concepts, ideas, and data openly on the Internet can result in intellectual property abuse, and excessive engagement in social media may cause a loss of reputation among

peers. However, when practiced skillfully, Public Science 2.0 is likely to inspire laypeople and researchers alike.

References and Notes

1. J. Teichmann, *Vom Bernstein zum Elektron* (3rd ed., Deutsches Museum, Munich, 1998).
2. A. Miller-Rushing, R. Primack, R. Bonney, *Front. Ecol. Environ.* **10**, 285 (2012).
3. www.rigb.org
4. A. Engel, transl., E. Schucking, consultant Ed., *The Collected Papers of Albert Einstein, Volume 7: The Berlin Years: Writings, 1918–1921, English translation supplement* (Princeton Univ. Press, Princeton, NJ, 2002). p.160 and p.261.
5. A. Einstein, *Über die spezielle und die allgemeine Relativitätstheorie (Gemeinverständlich)* (Vieweg, Braunschweig, 1917).
6. A. Einstein, *Verehrte An- und Abwesende. Original audio recordings 1921–1951* (Supposé, Cologne, 2003), CD 1; see also www.einstein-website.de/z_biography/credo.html.
7. P. Weingart, in *Wissenschaft und Hochschulbildung im Kontext von Wirtschaft und Medien*, B. Hölscher, J. Suchanek, Eds. (Springer VS, Wiesbaden, 2011), p. 54. Quote translated by Tom Harris.
8. www.realclimate.org
9. www.scilogs.de/wblogs/blog/kimalounge
10. H. M. Bik, M. C. Goldstein, *PLoS Biol.* **11**, e1001535 (2013).
11. J. Silvertown, *Trends Ecol. Evol.* **24**, 467 (2009).
12. Entladung einer Leidener Flasche über eine Menschenkette, from *Dictionnaire universel de mathématique et de physique*, Paris 1753, volume 1.

Acknowledgments: C.K. founded SciLogs in 2007. B.L. was managing editor of scienceblogs.de when it was launched.

10.1126/science.1245848

PLANT SCIENCE

Fine-Tuning Photosynthesis

Jean-David Rochaix

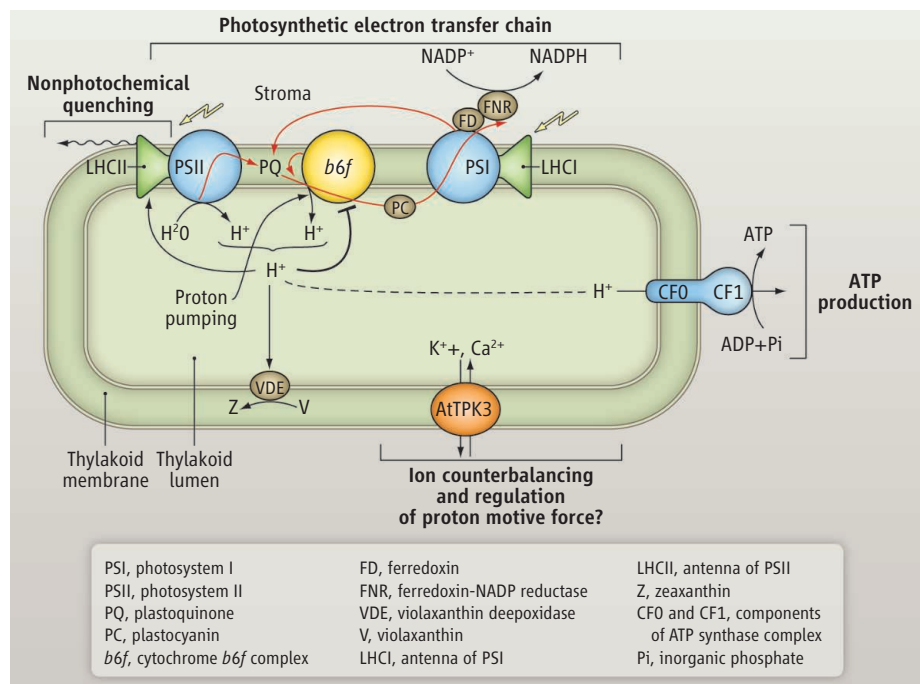
A potassium channel helps to fine-tune the photosynthetic machinery of plants to changes in environmental conditions.

Most biochemical reactions in living cells are fueled by adenosine triphosphate (ATP) and/or the reducing agent reduced nicotinamide adenine dinucleotide phosphate (NADPH). In plant cells, these components are generated in mitochondria and chloroplasts through the respiratory and photosynthetic electron transfer chains. Nearly 50 years ago, Mitchell (1) proposed a unifying mechanism through which the respiratory and photosynthetic electron transfer reactions are coupled to proton pumping across the inner mito-

chondrial and thylakoid membranes; the thylakoid membranes are located within chloroplasts and contain the protein-pigment complexes that catalyze the primary reactions of photosynthesis (see the figure). This proton movement generates a transmembrane electrochemical gradient called the proton motive force. On page 114 of this issue, Carretero *et al.* (2) show how a two-pore potassium channel helps to regulate the proton motive force.

The proton motive force consists of an electric potential ($\Delta\Psi$) and an osmotic component (ΔpH), both of which are used by the ATP synthase to produce ATP (see the figure) (3). ΔpH also regulates electron transfer between plastoquinol and the cytochrome

Department of Molecular Biology and Department of Plant Biology, University of Geneva, 30 Quai Ernest Ansermet, Geneva 4, 1211 Geneva, Switzerland. E-mail: jean-david.rochaix@unige.ch



Regulation of photosynthesis. Electron transfer along the photosynthetic electron transport chain leads to the formation of NADPH and is coupled to proton pumping from the stroma to the thylakoid lumen and the formation of a proton motive force across the thylakoid membrane, consisting of the electric potential $\Delta\Psi$ and an osmotic component ΔpH . The proton motive force is used by the ATP synthase to produce ATP. The proton gradient regulates electron flow, and the resulting low pH in the lumen induces conformational changes in the photosystem II antenna and activates enzymes of the xanthophyll cycle (VDE), which together promote energy-dependent nonphotochemical quenching, a process that decreases photosynthesis and prevents photodamage under high light or CO_2 limitation. AtTPK3, the two-pore potassium channel identified by Carraretto *et al.*, may help to regulate the partitioning of the proton motive force between $\Delta\Psi$ and ΔpH .

b_6f complex, the rate-limiting step in the photosynthetic electron transport chain (4). Furthermore, ΔpH triggers the dissipation of excess absorbed light energy as heat at the expense of photochemistry and electron flow, a process called energy-dependent nonphotochemical quenching (5) that diminishes the formation of reactive oxygen species and thereby provides photoprotection. The proton motive force is adjusted in response to environmental changes and plays a pivotal role in the acclimation of the photosynthetic machinery by balancing photochemical efficiency and photoprotection. It remains unclear how the partitioning between $\Delta\Psi$ and ΔpH is regulated.

Thylakoid membranes consist of regions of stacked membranes that are connected by single stromal lamellae. Carraretto *et al.* have identified a two-pore potassium channel, AtTPK3, in these lamellae. The channel belongs to the TPK (tandem-pore K^+ channel) family in *Arabidopsis* (6). Among the family members, only AtTPK3 is localized in the thylakoid membranes. It appears to regulate the proton motive force through ion counterbalancing. Carraretto *et al.* show that recombinant AtTPK3 displays K^+ -selective channel

activity sensitive to Ca^{2+} and H^+ . Moreover, plants silenced for TPK3 grew more slowly and their thylakoid membrane organization was altered when they were grown under moderate irradiance. Under these conditions, the authors observed a decrease in ΔpH and a compensatory increase in $\Delta\Psi$, with little change in the total proton motive force. Moreover, the altered thylakoid membrane folding was accompanied by changes in thylakoid protein phosphorylation, and CO_2 assimilation and nonphotochemical quenching were diminished.

These results indicate that AtTPK3 mediates electroneutrality during light-induced lumen acidification through ion counterbalancing. For each proton pumped into the thylakoid lumen space, a K^+ ion or another cation is extruded to the stromal phase. Because of the proton-buffering capacity of the thylakoid lumen, ΔpH is not expected to increase further, and this will prevent the inactivation of lumenal enzymes. In this way, AtTPK3 could play an important role in controlling the size of the proton motive force and modulating its composition, which is critical for the proper utilization and dissipation of absorbed light excitation energy.

The results raise the question of whether AtTPK3 plays a regulatory role in this process. If so, it remains to be shown how its ion channel activity is regulated and how it senses changes in environmental conditions. However, it cannot be excluded that other factors besides AtTPK3 regulate the partitioning of the proton motive force in $\Delta\Psi$ and ΔpH .

Earlier studies with isolated thylakoids revealed a large proton gradient across the thylakoid membrane with a stromal pH of 7.5 to 7.8 and a lumenal pH of 4.5 to 4.8. This observation suggested that counterions such as Cl^- , Mg^{2+} , and K^+ annihilate all of $\Delta\Psi$, with the result that the proton motive force consists only of ΔpH . However, such conditions are difficult to reconcile with the in vivo biochemical properties of the photosynthetic machinery (7, 8). The identification of AtTPK3 by Carraretto *et al.* provides new clues as to how a variable part of the proton motive force can be stored as $\Delta\Psi$, thereby limiting the acidity of the thylakoid lumen under optimal growth conditions. Recent in vitro and in vivo measurements of light-driven electrochromic shifts indicate indeed that up to half the proton motive force can be stored as $\Delta\Psi$ (9, 10). A lower lumenal pH would only occur under certain stresses, such as high light and CO_2 limitation (7).

The picture that emerges from these studies is that proton motive force parsing into $\Delta\Psi$ and ΔpH is a key process for fine-tuning the photosynthetic machinery to changes in environmental conditions. This proton motive force partitioning is intimately linked with plastid ion transport and ion homeostasis, which are poorly understood. Altering the amounts of counterions in the stroma could be one way to modulate the $\Delta\Psi$ and ΔpH fractions of the proton motive force, which would in turn affect nonphotochemical quenching and electron flow. In this respect, the identification of AtTPK3 is an important step toward a full understanding of the regulation and flexibility of the photosynthetic machinery.

References

1. P. Mitchell, *Biol. Rev. Camb. Philos. Soc.* **41**, 445 (1966).
2. I. Carraretto *et al.*, *Science* **342**, 114 (2013).
3. S. Fischer, P. Gräber, *FEBS Lett.* **457**, 327 (1999).
4. A. B. Hope, P. Valente, D. B. Matthews, *Photosynth. Res.* **42**, 111 (1994).
5. K. K. Niyogi, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 333 (1999).
6. D. Becker *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15621 (2004).
7. D. M. Kramer *et al.*, *Trends Plant Sci.* **8**, 27 (2003).
8. C. A. Sacksteder, A. Kanazawa, M. E. Jacoby, D. M. Kramer, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 14283 (2000).
9. T. J. Avenson, J. A. Cruz, D. M. Kramer, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5530 (2004).
10. J. A. Cruz, C. A. Sacksteder, A. Kanazawa, D. M. Kramer, *Biochemistry* **40**, 1226 (2001).

10.1126/science.1244943



A Looser Clock to Cure Jet Lag

Michael H. Hastings

Science **342**, 52 (2013);

DOI: 10.1126/science.1245474

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/52.full.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/52.full.html#related>

This article **cites 12 articles**, 4 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/52.full.html#ref-list-1>

PHYSIOLOGY

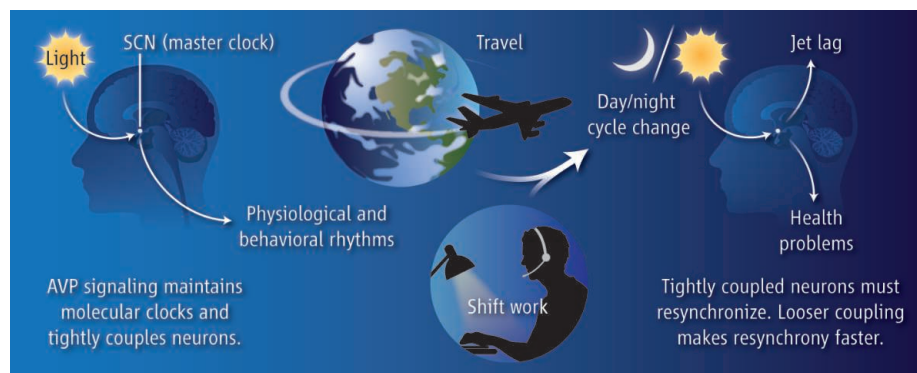
A Looser Clock to Cure Jet Lag

Michael H. Hastings

Jet lag is a blessing to circadian biologists because the disruption of mental and physical well-being immediately highlights the importance of our internal “body clock.” It is also a curse because jet lag has so far eluded attempts at a cure. On page 85 of this issue, Yamaguchi *et al.* (1) show that mice lacking receptors for the neuropeptide arginine vasopressin (AVP) are resistant to jet lag, providing new hope of overcoming this modern malaise. Not only may this help us recover from symptoms of jet lag, but it should also help unravel the neural circuit that sets the tempo to our lives.

Daily rhythms of behavior and physiology, most obviously sleep and wakefulness, adapt us to the cyclical day-night environment. These rhythms are generated by a cell-autonomous clock, with an intrinsic period of approximately 1 day. The clock is composed of interlocked molecular loops within which the activity of “clock” genes is suppressed by their protein products (2). Because this negative feedback occurs with a delay of several hours (3), the loops spontaneously oscillate with a circadian period. Although this timer is expressed within most, if not all, cells (4), this body-wide system is coordinated by the suprachiasmatic nucleus (SCN) of the hypothalamus, the “master clock” of the brain (see the figure). The SCN has two special properties: It receives a dedicated retinal innervation, making it the only part of the circadian axis able to synchronize to the light-dark cycle (5); and, the ~10,000 SCN neurons can synchronize their molecular feedback loops, one to another, to generate at the circuit level a far more robust, sustained, and precise circadian output than other tissues (6). This enhancement relies on paracrine signaling between neurons (7), involving the neuropeptides arginine vasopressin (AVP), vasoactive intestinal peptide (VIP), and gastrin-releasing peptide, and their cognate receptors (8).

The distinctive properties of the SCN—sensitivity to lighting cycles and stable pace-making arising from tight molecular coupling—are reflected in the essential features of jet lag. Our circadian system can sense and realign to a shifted cycle of light and dark-



Readjustment. Signaling by arginine vasopressin (AVP) in the SCN maintains the molecular clocks and tightly couples neurons in the “master clock,” thereby controlling the rhythmicity of human physiology. Disruption of one’s normal day-night cycle (by long-distance travel, for example) disrupts this synchronicity, which can lead to the symptoms of jet lag. In mice, preventing AVP signaling allows the animals to experience large changes in their normal day-night phases without exhibiting jet-lag behaviors. This may be due to a looser coupling among AVP neurons that allows the entire ensemble to adapt more rapidly in the face of environmental change.

ness, but its intrinsic stability confers inertia, slowing the realignment. The temporary loss of internal circadian synchrony as readjustment progresses causes symptoms that include sleep disturbance and dysphoria, and the rule of thumb, for humans flying between time zones (and for mice subjected to an acute shift of lighting schedule), is that it takes about 1 day to readjust for every 1-hour change in environmental time. Yamaguchi *et al.* find that mice genetically deficient in AVP receptors (types V1a and V1b, both highly expressed in the mouse SCN) readjust their behavior almost immediately to 8-hour advances or delays of the lighting regime. This is equivalent to flying between Los Angeles and London without the accompanying “red-eye.” And it is not solely behavior that springs forward and backward: Circadian rhythms of gene expression in the SCN, liver, and kidney all readjust with astonishing rapidity. Importantly, pharmacological blockade of AVP receptors in wild-type mice recapitulates the effect of the mutations.

How might loss of AVP receptors leave clock sensitivity to retinal input intact, but reduce clock inertia, and is it specific to AVP signaling? Mice lacking VIP or its receptor show a phenotype comparable to that of AVP-deficient mice insofar as behavioral readjustment immediately follows a shift in lighting cycle. But this is misleading because VIP-deficient mice lack an effective clock: Their

A neuropeptide that signals in the brain’s master circadian clock may be the key to overcoming the symptoms of malaise associated with jet lag.

SCN neurons are unable to synchronize and their circadian feedback loops wind down. Nevertheless, being mice, they suppress activity in the light, giving the false impression of a rapidly shifting clock. The startling feature of the study by Yamaguchi *et al.* is that the AVP-deficient mice have perfectly good clocks, so that under continuous darkness they show pronounced circadian rhythms of behavior, body temperature, and gene expression (in contrast to VIP-deficient mice). Further, SCN slices from the AVP-deficient mice sustain beautiful cellular circadian rhythms of bioluminescent gene expression, synchronized across the circuit. So here is the unexpected paradox: an SCN that is both a precise time-keeper (which requires stability and coupling) and yet capable of making extremely large phase-jumps.

To probe this further, Yamaguchi *et al.* perturbed the intracellular loops by blocking protein synthesis with cycloheximide. In the normal SCN, this treatment temporarily suspends the intracellular oscillations of gene expression by interrupting the protein-mediated negative feedback. Nevertheless, after removal of cycloheximide, the molecular loops recommence and the uncoupled cells rapidly resynchronize, establishing the original circuit-level phase order and amplitude of circadian gene expression. In the AVP-deficient mice, however, the circuits had serious difficulties in reassembling after

Division of Neurobiology, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK. E-mail: mha@mrc-lmb.cam.ac.uk

cycloheximide withdrawal, indicating that the “gravitational pull” between neurons is compromised. Yamaguchi *et al.* conclude that this looser coupling between the cells of AVP-deficient SCN is sufficient to maintain normal circadian function in a steady state, but makes the SCN more responsive to extreme perturbation because the cells are no longer held back by tight phase-locking across the circuit.

Whether it is simply a happy accident that loss of AVP signals leads to this novel state, or there is some deeper SCN design principle is unclear. More widely, the study by Yamaguchi *et al.* shows how neuropeptidergic signaling confers circuit-level properties on a population of neurons. In itself, neuropeptidergic generation of emergent properties is not unusual, having been especially well characterized in invertebrate systems (9). What is particular about the SCN is the time frame involved, with neuropeptidergic signals encoding very precise information over a span of hours and days, rather than milliseconds and minutes. Given that the circadian phenotype is pronounced and

stereotypical, and in cell culture it emerges from a circuit of less than 10,000 neurons (which is the approximate number of neurons of some invertebrate nervous systems), it should be possible to perform a comparable cellular and molecular analysis of the system that defines our biological time. Beyond that, identifying how neuropeptidergic cues from the SCN maintain the circadian coherence across the brain, which is essential for normal sleep and wakefulness, will provide a new level of understanding to the greatest emergent property of all: states of consciousness.

If developing AVP receptor antagonists did help us to jet ever more frantically about the planet, it would affect only a small minority of people. In the context of clocks and public health, however, there is a more insidious threat. Epidemiology shows that rotational shift work is a killer, increasing risks of cancer, and cardiovascular and metabolic diseases (10, 11). If the 24/7 society is here to stay, helping shift-workers adjust more rapidly to their schedules by working with, rather than against, their SCN must

be a good thing. Added to that, sleep disorders are a growing problem, for both normal aging and various dementias, and exploiting AVP signaling to tighten up the aging clock and its control over sleep may prove a useful tool. But there remains a final thought. After 4 billion years of circadian evolution (12), can you really cheat on circadian time?

References

1. Y. Yamaguchi, *Science* **342**, 85 (2013).
2. S. M. Reppert, D. R. Weaver, *Nature* **418**, 935 (2002).
3. N. Koike *et al.*, *Science* **338**, 349 (2012).
4. S. H. Yoo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5339 (2004).
5. T. M. Schmidt, S. K. Chen, S. Hattar, *Trends Neurosci.* **34**, 572 (2011).
6. A. C. Liu *et al.*, *Cell* **129**, 605 (2007).
7. E. S. Maywood, J. E. Chesham, J. A. O'Brien, M. H. Hastings, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14306 (2011).
8. M. Brancaccio, E. S. Maywood, J. E. Chesham, A. S. Loudon, M. H. Hastings, *Neuron* **78**, 714 (2013).
9. C. I. Bargmann, E. Marder, *Nat. Methods* **10**, 483 (2013).
10. G. M. Monsees, P. Kraft, S. E. Hankinson, D. J. Hunter, E. S. Schernhammer, *Int. J. Cancer* **131**, 2547 (2012).
11. M. H. Hastings, A. B. Reddy, E. S. Maywood, *Nat. Rev. Neurosci.* **4**, 649 (2003).
12. R. S. Edgar *et al.*, *Nature* **485**, 459 (2012).

10.1126/science.1245474

SOCIAL SCIENCE

A Risky Science Communication Environment for Vaccines

Dan M. Kahan

Controversy over childhood vaccinations is an instance of what might be styled the “science communication problem”—the failure of compelling scientific evidence to resolve public dispute over risks and similar facts (1). This problem itself has been the focus of scientific study since the 1970s, when psychologists began to investigate the divergence between expert and public opinion on nuclear power. Indeed, the science of science communication that this body of work comprises can now be used not just to explain controversy over risk but also to predict, manage, and in theory avoid conditions likely to trigger it. The example of childhood vaccinations illustrates these points—and teaches an important practical lesson.

One recurring source of risk controversy is a dynamic known as “cultural cognition.” Both to avoid dissonance and to protect their ties to others, individuals face a strong psy-

chic pressure to conform their perceptions of risk to those that distinguish their group from competing ones—a bias in reasoning that can actually intensify as the public becomes more science literate (2).

A major factor in the dispute over climate change, cultural cognition has contributed



Shots of controversy. Conflict over childhood vaccinations reflect the inadequate attention given to understanding factors in the science communication environment that influence the public understanding of science.

Neglecting the science of science communication puts the value of decision-relevant science at risk.

to controversy over at least one childhood vaccine as well. In 2006, the U.S. Centers for Disease Control (CDC) recommended universal immunization of adolescent girls against the human papilloma virus (HPV), which is sexually transmitted and causes cervical cancer, but political dispute blocked

legislative mandates in every state but one. Experimental evidence showed that individuals tended to selectively credit information relating to the vaccine's risks and benefits in patterns reflecting their cultural predispositions (one perceived risk was that vaccination would lead to the engagement of unsafe sex). The resulting polarization was amplified when individuals were exposed to cues—whether explicit, such as news reports (3), or tacit, such as fictional advocates of varying appearances (4)—suggesting the vaccine was a focus of group conflict.



A Risky Science Communication Environment for Vaccines

Dan M. Kahan

Science **342**, 53 (2013);

DOI: 10.1126/science.1245724

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/53.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/03/342.6154.53.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/53.full.html#related>

This article **cites 11 articles**, 1 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/53.full.html#ref-list-1>

This article has been **cited by** 1 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/342/6154/53.full.html#related-urls>

cycloheximide withdrawal, indicating that the “gravitational pull” between neurons is compromised. Yamaguchi *et al.* conclude that this looser coupling between the cells of AVP-deficient SCN is sufficient to maintain normal circadian function in a steady state, but makes the SCN more responsive to extreme perturbation because the cells are no longer held back by tight phase-locking across the circuit.

Whether it is simply a happy accident that loss of AVP signals leads to this novel state, or there is some deeper SCN design principle is unclear. More widely, the study by Yamaguchi *et al.* shows how neuropeptidergic signaling confers circuit-level properties on a population of neurons. In itself, neuropeptidergic generation of emergent properties is not unusual, having been especially well characterized in invertebrate systems (9). What is particular about the SCN is the time frame involved, with neuropeptidergic signals encoding very precise information over a span of hours and days, rather than milliseconds and minutes. Given that the circadian phenotype is pronounced and

stereotypical, and in cell culture it emerges from a circuit of less than 10,000 neurons (which is the approximate number of neurons of some invertebrate nervous systems), it should be possible to perform a comparable cellular and molecular analysis of the system that defines our biological time. Beyond that, identifying how neuropeptidergic cues from the SCN maintain the circadian coherence across the brain, which is essential for normal sleep and wakefulness, will provide a new level of understanding to the greatest emergent property of all: states of consciousness.

If developing AVP receptor antagonists did help us to jet ever more frantically about the planet, it would affect only a small minority of people. In the context of clocks and public health, however, there is a more insidious threat. Epidemiology shows that rotational shift work is a killer, increasing risks of cancer, and cardiovascular and metabolic diseases (10, 11). If the 24/7 society is here to stay, helping shift-workers adjust more rapidly to their schedules by working with, rather than against, their SCN must

be a good thing. Added to that, sleep disorders are a growing problem, for both normal aging and various dementias, and exploiting AVP signaling to tighten up the aging clock and its control over sleep may prove a useful tool. But there remains a final thought. After 4 billion years of circadian evolution (12), can you really cheat on circadian time?

References

1. Y. Yamaguchi, *Science* **342**, 85 (2013).
2. S. M. Reppert, D. R. Weaver, *Nature* **418**, 935 (2002).
3. N. Koike *et al.*, *Science* **338**, 349 (2012).
4. S. H. Yoo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5339 (2004).
5. T. M. Schmidt, S. K. Chen, S. Hattar, *Trends Neurosci.* **34**, 572 (2011).
6. A. C. Liu *et al.*, *Cell* **129**, 605 (2007).
7. E. S. Maywood, J. E. Chesham, J. A. O'Brien, M. H. Hastings, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14306 (2011).
8. M. Brancaccio, E. S. Maywood, J. E. Chesham, A. S. Loudon, M. H. Hastings, *Neuron* **78**, 714 (2013).
9. C. I. Bargmann, E. Marder, *Nat. Methods* **10**, 483 (2013).
10. G. M. Monsees, P. Kraft, S. E. Hankinson, D. J. Hunter, E. S. Schernhammer, *Int. J. Cancer* **131**, 2547 (2012).
11. M. H. Hastings, A. B. Reddy, E. S. Maywood, *Nat. Rev. Neurosci.* **4**, 649 (2003).
12. R. S. Edgar *et al.*, *Nature* **485**, 459 (2012).

10.1126/science.1245474

SOCIAL SCIENCE

A Risky Science Communication Environment for Vaccines

Dan M. Kahan

Controversy over childhood vaccinations is an instance of what might be styled the “science communication problem”—the failure of compelling scientific evidence to resolve public dispute over risks and similar facts (1). This problem itself has been the focus of scientific study since the 1970s, when psychologists began to investigate the divergence between expert and public opinion on nuclear power. Indeed, the science of science communication that this body of work comprises can now be used not just to explain controversy over risk but also to predict, manage, and in theory avoid conditions likely to trigger it. The example of childhood vaccinations illustrates these points—and teaches an important practical lesson.

One recurring source of risk controversy is a dynamic known as “cultural cognition.” Both to avoid dissonance and to protect their ties to others, individuals face a strong psy-

chic pressure to conform their perceptions of risk to those that distinguish their group from competing ones—a bias in reasoning that can actually intensify as the public becomes more science literate (2).

A major factor in the dispute over climate change, cultural cognition has contributed



Shots of controversy. Conflict over childhood vaccinations reflect the inadequate attention given to understanding factors in the science communication environment that influence the public understanding of science.

Neglecting the science of science communication puts the value of decision-relevant science at risk.

to controversy over at least one childhood vaccine as well. In 2006, the U.S. Centers for Disease Control (CDC) recommended universal immunization of adolescent girls against the human papilloma virus (HPV), which is sexually transmitted and causes cervical cancer, but political dispute blocked

legislative mandates in every state but one. Experimental evidence showed that individuals tended to selectively credit information relating to the vaccine's risks and benefits in patterns reflecting their cultural predispositions (one perceived risk was that vaccination would lead to the engagement of unsafe sex). The resulting polarization was amplified when individuals were exposed to cues—whether explicit, such as news reports (3), or tacit, such as fictional advocates of varying appearances (4)—suggesting the vaccine was a focus of group conflict.

The same insights that explain the controversy over the HPV vaccine, however, imply that it need not have occurred. It was likely inevitable that people of opposing cultural orientations would react divergently to a high-profile campaign to enact legislation mandating vaccination of 11- to 12-year-old girls for a sexually transmitted disease. Yet there was nothing inevitable about the HPV vaccine being publicly introduced in a manner so likely to generate cultural conflict.

Merck, the manufacturer of the HPV vaccine Gardasil, sought approval from the U.S. Food and Drug Administration (FDA) through the agency's fast-track review process, which is reserved for treatments of serious diseases—in this case, for a female-only vaccine for cervical cancer. After approval, the company sponsored a nationwide lobbying campaign directed at state legislatures to add the vaccine to the schedule of immunizations required for school enrollment. These were profit-driven choices (5), aimed at enabling Merck to establish a dominant market position for Gardasil before GlaxoSmithKline could secure approval for its rival product, Cervarix. If Gardasil had not been fast-tracked, the FDA would have approved both Gardasil and Cervarix for boys and girls only 3 years later. At that point, both vaccines would have become available immediately even without mandates through private insurance and a host of programs designed to assure universal access to childhood vaccines.

Had the HPV vaccine taken this path, it would have followed the uneventful course that marked introduction of the hepatitis B virus (HBV) vaccine into the U.S. public health system. Hepatitis B, like HPV, is sexually transmitted and causes cancer (6). The CDC endorsed universal childhood HBV vaccination—for boys and girls, a much less jarring proposal—in the 1990s. There was no political controversy. Rather, states steadily added the HBV vaccine to mandatory vaccination schedules through the customary mechanism—not high-profile legislative enactments, but guidelines routinely promulgated by public health administrators operating outside the political realm (7). The HPV vaccine might well have been handled in the same way had it not been introduced as a mandatory, girls-only shot for a sexually transmitted disease in a nationwide legislative campaign [(religious groups were not opposed to FDA approval of the vaccine per se (8)]. But even more important, parents' first exposure to information on HPV

vaccine would not have been from partisan news outlets. Rather, they would have learned about the vaccine from their pediatricians. The same studies reporting that culturally diverse individuals would polarize if exposed to cues of group conflict showed that in the absence of such cues, members of all groups could have been expected to trust expert advice (4, 5). Parents do trust their pediatricians on the HBV vaccine, which retained coverage of 90% of children during the period when HPV mandates were being debated in state legislatures (9, 10). The rate for completing the HPV immunization series now stands at an anemic 33% for adolescent girls, and 7% for boys (11).

Many experts and medical groups warned that the HPV vaccine was being introduced in a manner likely to engulf it in controversy (5, 8). Their concerns were not rejected. They were simply never considered. There was and remains no process in the FDA or the CDC for making evidence-based assessments of the potential impact of their procedures on the myriad everyday channels through which the public becomes apprised of decision-relevant science.

Similar inattention to the quality of the science communication environment leaves other childhood vaccines vulnerable to controversy too. In the United Kingdom, childhood vaccination rates are only now recovering from the scare induced by the now-discredited 1998 study of Dr. Andrew Wakefield linking the measles, mumps, and rubella (MMR) vaccine to autism. By contrast, the United States experienced no such decline—vaccination rates for MMR, pertussis, and polio have been at or above 90% (the target level) for over a decade, and the proportion of children receiving no vaccinations has remained below 1% (9, 10). But there are enclaves, some populated by strident opponents of mandatory immunization, where vaccination rates fall dangerously short of the national average, and where local outbreaks of childhood diseases periodically occur. Evidence-informed risk communication strategies are essential to identify and counteract any influence that could cause ungrounded fears of vaccines to spread to the general population.

Ironically, one such influence is empirically uninformed risk communication. The media and advocacy groups routinely lament a “growing distrust of vaccinations” (12) and a resulting “erosion in immunization rates” (13), claims belied by CDC statistics. Emphatic assertions that a technology poses

no danger can actually enhance its perceived riskiness (14). In addition, people tend to contribute voluntarily to public goods—such as herd immunity—when they believe that others are doing so but refrain when they perceive widespread free-riding (15). Thus, misleadingly implying that increasing numbers of parents are fearfully refusing vaccination could create exactly such fear and resistance.

Also ill-advised is a popular trope that links resistance to childhood vaccination with disbelief in evolution and doubt of climate change as instances of public “distrust in science.” Critics of mandatory vaccination are small in number and their hostility to vaccines is generally unshared by the majority of the population. Positions on evolution and climate change, by contrast, are highly charged symbols for large cultural groups. Finding popular discourse with the claim that childhood vaccination is part of the same package of partisan stances as these issues (16) needlessly risks provoking the same cultural cognition dynamics that impeded reasoned public engagement with the HPV vaccine.

Empirically uniformed and counterproductive risk communication is the inevitable by-product of the absence of a systematic, evidence-based alternative. Decades of study show that the sources of public controversy over decision-relevant science are numerous and diverse. There is, however, a single factor that connects them: The failure of democratic societies to use scientific knowledge to protect the science communication environment from influences that prevent citizens from recognizing that decision-relevant science contributes to their well-being.

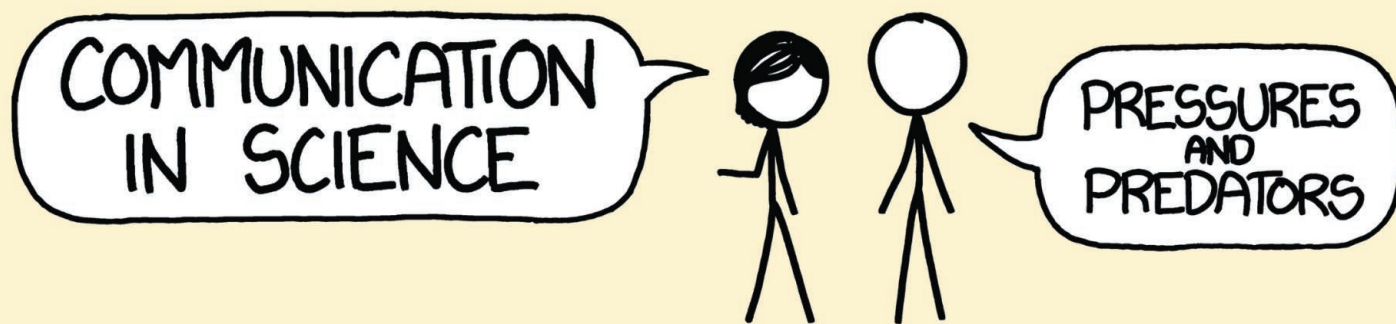
References

1. P. Slovic, *The Feeling of Risk* (Earthscan, Washington, DC, 2010).
2. D. Kahan et al., *Nat. Clim. Change* **2**, 732 (2012).
3. S. E. Gollust et al., *Health Aff.* **29**, 2041 (2010).
4. D. M. Kahan et al., *Law Hum. Behav.* **34**, 501 (2010).
5. L. O. Gostin, C. D. DeAngelis, *JAMA* **297**, 1921 (2007).
6. CDC, *Morb. Mortal. Wkly. Rep.* **54**, RR-16 (2005).
7. Immunization Action Coalition, www.immunize.org/laws/hepb.asp.
8. J. Colgrove, *N. Engl. J. Med.* **355**, 2389 (2006).
9. CDC, *Morb. Mortal. Wkly. Rep.* **57**, 961 (2008).
10. CDC, *Morb. Mortal. Wkly. Rep.* **62**, 733 (2013).
11. CDC, *Morb. Mortal. Wkly. Rep.* **62**, 685 (2013).
12. P. Devorak, *Washington Post*, 18 October 2011, http://articles.washingtonpost.com/2011-10-18/local/35280317_1_andrew-wakefield-vaccines-and-autism-hpv-vaccine.
13. National Public Radio, *Talk of the Nation*, 7 January 2011, www.npr.org/2011/01/07/132740175/paul-offit-on-the-anti-vaccine-movement.
14. D. J. Lanksa, *J. Public Health Policy* **19**, 160 (1998).
15. J. C. Hershey et al., *Organ. Behav. Hum. Decis. Process.* **59**, 177 (1994).
16. A. Frank, “Welcome to the age of denial,” *New York Times*, 21 August 2013, p. A27.

Online

sciencemag.org

Podcast interview with author Dan M. Kahan (http://scim.ag/ed_6154).



INTRODUCTION

Scientific Discourse: Buckling at the Seams

THOMAS EDISON BUILT AN EMPIRE ON HIS 1093 PATENTS. BUT ONE INNOVATION he considered a failure has had a lasting impact on how scientists communicate. He bankrolled the startup of *Science*, among the first general science journals, which debuted on 3 July 1880 with a bland description of the U.S. Naval Observatory and a cover plastered with classified ads. *Science* faltered at first, but in the end it thrived, and so did scientific discourse.

The mid-20th century saw a key innovation, the anonymous referee. This mechanism depends on trust, in both the integrity of submissions and in peer reviewers. That trust is being tested by a disruptive change in scientific communication: open access. Unlike “traditional” journals, which rely largely on subscription revenue, many open-access publications earn their daily bread through publication fees from authors. Profit is linked to volume, seemingly boundless on the Internet.

Although the open-access world includes many legitimate journals, abuse is prevalent, as a *Science* investigation has found. Over the past 10 months, contributing correspondent John Bohannon submitted faux papers with blatant scientific flaws to 304 open-access journals (p. 60). More than half accepted the paper.

Granted, some “traditional” print publications might have fallen for our hoax, too. But with open-access journals proliferating, debate is needed about how to ensure the credibility of scientific literature. Open-access pioneer Vitek Tracz believes that anonymous peer review is “sick and collapsing under its own weight.” As a remedy, Tracz has launched a new open-access journal in which reports—including all supporting data—are reviewed after they are posted online (p. 66). The findings and ex post facto reviews become a living document that proponents say will offer a more nimble forum for revising knowledge as it accumulates.

As the number of published papers (and the cost of doing research) grows, there is an increasing need to predict impact; for a novel approach, see the Report by Wang *et al.* (p. 127) and an accompanying Commentary (Evans, p. 44).

The ability to publish papers and their underlying data in full on the Internet opens new possibilities to showcase the neglected stepchild of scientific publishing: negative results. In the past, data revealing that a drug has not lived up to its promise, for example, often failed to see the light of day (p. 68). But now they may find a niche in the limitless library of the Internet.

On the other hand, disseminating certain scientific information could pose a threat to safety and security. The recent debate over whether to publish influenza gain-of-function studies illustrates the conundrum (p. 70). Scientists in industry, too, are struggling to define the limits of openness when communicating proprietary research, and whether some kinds of patents may actually squelch innovation (p. 72).

In the face of changes driven by the Internet, one form of communication is surprisingly resilient. By and large, scientists are unwilling to forgo the rite of annual meetings, where they gather to argue about new research, network, and gossip (p. 74) and to draw inspiration from top presenters (p. 78). The vitality of the scientific meeting has given rise to a troubling cottage industry: meetings held more for profit than enlightenment (p. 76).

How the dramatic shifts in scientific communication will affect the culture of research and processes for academic advancement and funding is far from clear (Harley, p. 80). In the Commentary section, Könniker and Lugger (p. 49) notes that we have come full circle: Once again, science is becoming more of a public activity. Kahan (p. 53) describes how to use evidence effectively and what pitfalls should be avoided in communicating vaccine information to a wary public.

— RICHARD STONE AND BARBARA JASNY

CONTENTS

News

- 58 The Rise of Open Access
- 60 Who's Afraid of Peer Review?
- 66 The Seer of Science Publishing
- 68 The Power of Negative Thinking
- 70 Hey, You've Got to Hide Your Work Away
Cloak-and-Dagger Publishing
- 72 Lock Up the Genome, Lock Down Research?
- 74 The Annual Meeting: Improving What Isn't Broken
What's Lost When a Meeting Goes Virtual
Meetings That Flatter, but May Not Deliver
Great Presenters

Policy Forum

- 80 Scholarly Communication: Cultural Contexts, Evolving Models
D. Harley

See also Editorial p. 13; Perspectives pp. 44, 49, and 53; Report p. 127; Science Careers; and Podcast at www.sciencemag.org/special/scicomm

Please Take Our Survey!

We're eager for your thoughts on open-access publishing and invite you to participate in an online survey: <http://scim.ag/OA-Poll>. Results will appear next month in *Science*.

Science



HOW MUCH SCIENCE IS THERE?

SCIENTIFIC PUBLISHING HAS BEEN ACCELERATING—A NEW PAPER IS NOW PUBLISHED ROUGHLY EVERY 20 SECONDS. LET'S IMAGINE A BIBLIOGRAPHY LISTING *EVERY* SCHOLARLY PAPER EVER WRITTEN. HOW LONG WOULD IT BE?

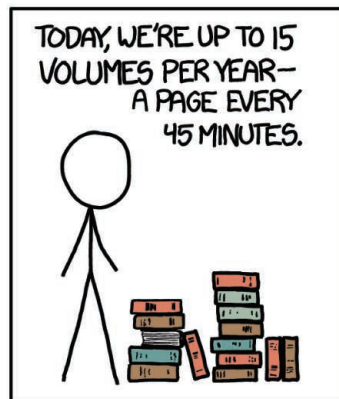
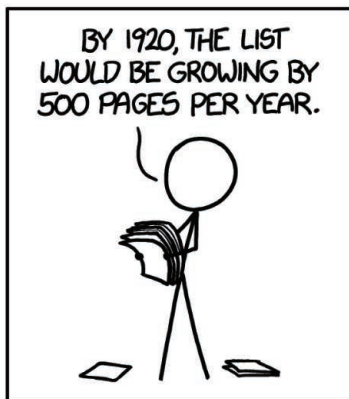
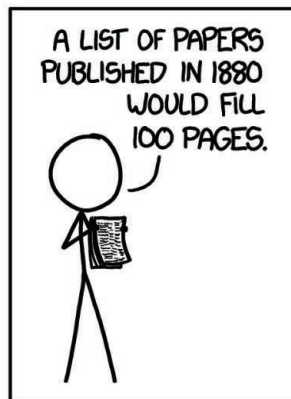
IF WE CAN FIT
140 CITATIONS
PER PAGE...



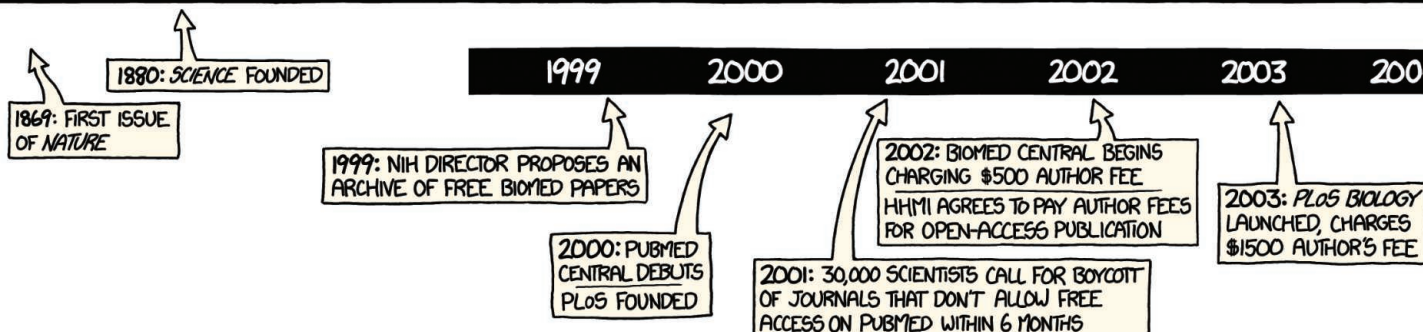
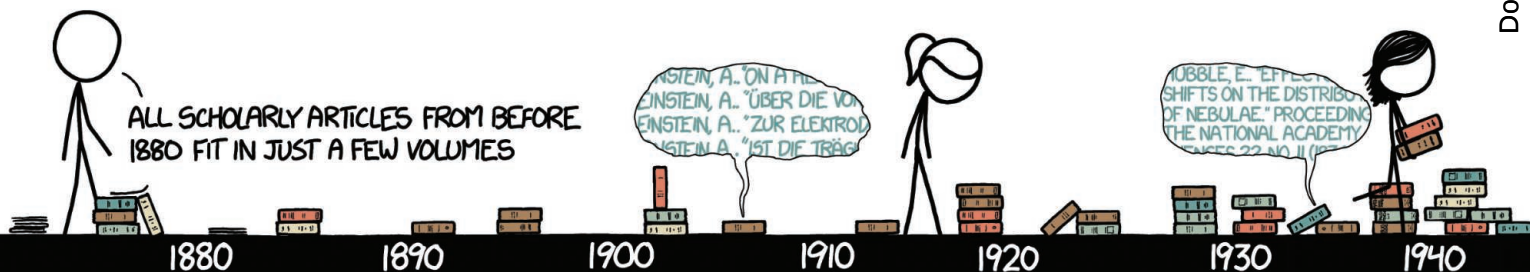
...1000 PAGES
PER BOOK...



...AND THEN WE START
STACKING BOOKS...

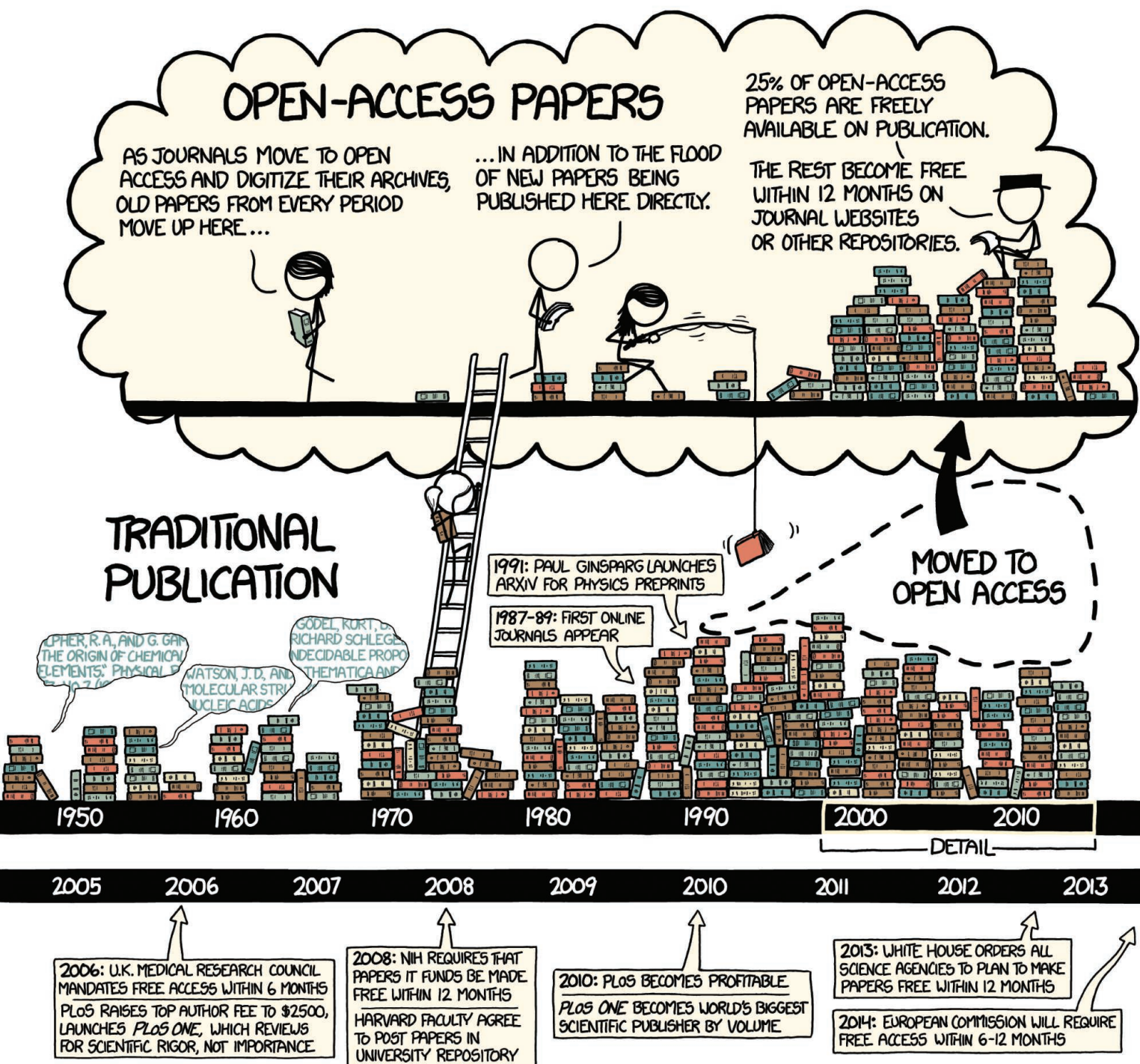


...THIS IS WHAT THE FULL LIST WOULD LOOK LIKE:



HOW OPEN IS IT?

SINCE THE ADVENT OF THE WEB, MUCH OF SCIENTIFIC PUBLISHING HAS BEEN MOVING TO OPEN ACCESS. ACCORDING TO SCIENCE-METRIX, OPEN ACCESS REACHED A "TIPPING POINT" AROUND 2011: MORE THAN 50% OF NEW RESEARCH IS NOW MADE AVAILABLE FREE ONLINE.



BY RANDALL MUNROE • REPORTING BY JOCELYN KAISER AND DAVID MALAKOFF



Who's Afraid of Peer Review?

A spoof paper concocted by *Science* reveals little or no scrutiny at many open-access journals

On 4 July, good news arrived in the inbox of Ocorrafoo Cobange, a biologist at the Wasee Institute of Medicine in Asmara. It was the official letter of acceptance for a paper he had submitted 2 months earlier to the *Journal of Natural Pharmaceuticals*, describing the anticancer properties of a chemical that Cobange had extracted from a lichen.

In fact, it should have been promptly rejected. Any reviewer with more than a high-school knowledge of chemistry and the ability to understand a basic data plot should have spotted the paper's shortcomings immediately. Its experiments are so hopelessly flawed that the results are meaningless.

I know because I wrote the paper. Ocorrafoo Cobange does not exist, nor does the Wasee Institute of Medicine. Over the past 10 months, I have submitted 304 versions of the wonder drug paper to open-access journals. More than half of the journals accepted the paper, failing to notice its fatal flaws. Beyond that headline result, the data from this sting operation reveal the contours of an emerging Wild West in academic publishing.

From humble and idealistic beginnings a decade ago, open-access scientific journals have mushroomed into a global industry, driven by author publication fees rather than traditional

subscriptions. Most of the players are murky. The identity and location of the journals' editors, as well as the financial workings of their publishers, are often purposefully obscured. But *Science's* investigation casts a powerful light. Internet Protocol (IP) address traces within the raw headers of e-mails sent by journal editors betray their locations. Invoices for publication fees reveal a network of bank accounts based mostly in the developing world. And the acceptances and rejections of the paper provide the first global snapshot of peer review across the open-access scientific enterprise.

One might have expected credible peer review at the *Journal of Natural Pharmaceuticals*. It describes itself as "a peer reviewed journal aiming to communicate high quality research articles, short communications, and reviews in the field of natural products with desired pharmacological activities." The editors and advisory board members are pharmaceutical science professors at universities around the world.

The journal is one of more than 270 owned by Medknow, a company based in Mumbai, India, and one of the largest open-access publishers. According to Medknow's website, more than

2 million of its articles are downloaded by researchers every month. Medknow was bought for an undisclosed sum in 2011 by Wolters Kluwer, a multinational firm headquartered in the Netherlands and one of the world's leading purveyors of medical information with annual revenues of nearly \$5 billion.

But the editorial team of the *Journal of Natural Pharmaceuticals*, headed by Editor-in-Chief Ilkay Orhan, a professor of pharmacy at Eastern Mediterranean University in Gazimagosa, Cyprus, asked the fictional Cobange for only superficial changes to the paper—different reference formats and a longer abstract—before accepting it 51 days later. The paper's scientific content was never mentioned. In an e-mail to *Science*, managing editor Mueen Ahmed, a professor of pharmacy at King Faisal University in Al-Hasa, Saudi Arabia, states that he will permanently shut down the journal by the end of the year. "I am really sorry for this," he says. Orhan says that for the past 2 years, he had left the journal's operation entirely to staff led by Ahmed. (Ahmed confirms this.) "I should've been more careful," Orhan says.

Acceptance was the norm, not the exception. The paper was accepted by journals hosted by industry titans Sage and Elsevier. The paper was accepted by journals published by prestigious academic institutions such as Kobe University in Japan. It was accepted by scholarly society journals. It was even accepted by journals for which the paper's topic was utterly inappropriate, such as the *Journal of Experimental & Clinical Assisted Reproduction*.

The rejections tell a story of their own. Some open-access journals that have been criticized for poor quality control provided the most rigorous peer review of all. For example, the flagship journal of the Public Library of Science, *PLOS ONE*, was the only journal that called attention to the paper's potential ethical problems, such as its lack of documentation about the treatment of animals used to generate cells for the experiment. The journal meticulously checked with the fictional authors that this and other prerequisites of a proper scientific study were met before sending it out for review. *PLOS ONE* rejected the paper 2 weeks later on the basis of its scientific quality.

Down the rabbit hole

The story begins in July 2012, when the *Science* editorial staff forwarded to me an e-mail thread from David Roos, a biologist at the University of Pennsylvania. The thread detailed the publication woes of Aline Noutcha, a biologist at the University of Port Harcourt in Nigeria. She had taken part in a research workshop run by Roos in Mali in January last year and had been trying to publish her study of *Culex quinquefasciatus*, a mosquito that carries West Nile virus and other pathogens.

Noutcha had submitted the paper to an open-access journal called *Public Health Research*. She says that she believed that publication would be free. A colleague at her university had just published a paper for free in another journal from the same publisher: Scientific & Academic Publishing Co. (SAP), whose website does not mention fees. After Noutcha's paper was accepted, she says, she was asked to pay a \$150 publication fee: a 50% discount because she is based in Nigeria. Like many developing world scientists, Noutcha does not have a credit card, and international bank transfers are complicated and costly. She eventually convinced a friend in the United States to pay a fee further reduced to

\$90 on her behalf, and the paper was published.

Roos complained that this was part of a trend of deceptive open-access journals "parasitizing the scientific research community." Intrigued, I looked into Scientific & Academic Publishing. According to its website, "SAP serves the world's research and scholarly communities, and aims to be one of the largest publishers for professional and scholarly societies." Its list includes nearly 200 journals, and I randomly chose one for a closer look. The *American Journal of Polymer Science* describes itself as "a continuous forum for the dissemination of thoroughly peer-reviewed, fundamental, international research into the preparation and properties of macromolecules." Plugging the text into an Internet search engine, I quickly found that portions had been cut and pasted from the website of the *Journal of Polymer Science*, a respected journal published by Wiley since 1946.

I began to wonder if there really is anything American about the *American Journal of Polymer Science*. SAP's website claims that the journal is published out of Los Angeles. The street address appears to be no more than the intersection of two highways, and no phone numbers are listed.

I contacted some of the people listed as the journal's editors and reviewers. The few who replied said they have had little contact with SAP. Maria Raimo, a chemist at the Institute of Chemistry and Technology of Polymers in Naples, Italy, had received an e-mail invitation to be a reviewer 4 months earlier. To that point, she had received a single paper—one so poor that "I thought it was a joke," she says.

Despite her remonstrations to the then-editor-in-chief, a person of unknown affiliation called David Thomas, the journal published the paper. Raimo says she asked to be removed from the masthead. More than a year later, the paper is still online and the journal still lists Raimo as a reviewer.

After months of e-mailing the editors of SAP, I finally received a response. Someone named Charles Duke reiterated—in broken English—that SAP is an American publisher based in California. His e-mail arrived at 3 a.m., Eastern time.

To replicate Noutcha's experience, I decided to submit a paper of my own to an SAP journal. And to get the lay of this shadowy publishing landscape, I would have to replicate the experiment across the entire open-access world.

The targets

The *Who's Who* of credible open-access journals is the Directory of Open Access Journals (DOAJ). Created 10 years ago by Lars Bjørnshauge, a library scientist at Lund University in Sweden, the DOAJ has grown rapidly, with about 1000 titles added last year alone. Without revealing my plan, I asked DOAJ staff members how journals make it onto their list. "The title must first be suggested to us through a form on our website," explained DOAJ's Linnéa Stenson. "If a journal hasn't published enough, we contact the editor or publisher and ask them to come back to us when the title has published more content." Before listing a journal, they review it based on information provided by the publisher. On 2 October 2012, when I launched my sting, the DOAJ contained 8250 journals and abundant metadata for each one, such as the name and URL of the publisher, the year it was founded, and the topics it covers.

It is a relief
to know that
our system
is working.

—PAUL PETERS,
HINDAWI



There is another list—one that journals fear. It is curated by Jeffrey Beall, a library scientist at the University of Colorado, Denver. His list is a single page on the Internet that names and shames what he calls “predatory” publishers. The term is a catchall for what Beall views as unprofessional practices, from undisclosed charges and poorly defined editorial hierarchy to poor English—criteria that critics say stack the deck against non-U.S. publishers.

Like Batman, Beall is mistrusted by many of those he aims to protect. “What he’s doing is extremely valuable,” says Paul Ginsparg, a physicist at Cornell University who founded arXiv, the preprint server that has become a key publishing platform for many areas of physics. “But he’s a little bit too trigger-happy.”

I asked Beall how he got into academic crime-fighting. The problem “just became too bad to ignore,” he replied. The population “exploded” last year, he said. Beall counted 59 predatory open-access publishers in March 2012. That figure had doubled 3 months later, and the rate has continued to far outstrip DOAJ’s growth.

To generate a comprehensive list of journals for my investigation, I filtered the DOAJ, eliminating those not published in English and those without standard open-access fees. I was left with 2054 journals associated with 438 publishers. Beall’s list, which I scraped from his website on 4 October 2012, named 181 publishers. The overlap was 35 publishers, meaning that one in five of Beall’s “predatory” publishers had managed to get at least one of their journals into the DOAJ.

I further whittled the list by striking off publishers lacking a general interest scientific journal or at least one biological, chemical, or medical title. The final list of targets came to 304 open-access publishers: 167 from the DOAJ, 121 from Beall’s list, and 16 that were listed by both. (Links to all the publishers, papers, and correspondence are available online at <http://scim.ag/OA-Sting>.)

The bait

The goal was to create a credible but mundane scientific paper, one with such grave errors that a competent peer reviewer should easily identify it as flawed and unpublishable. Submitting identical papers to hundreds of journals would be asking for trouble. But the papers had to be similar enough that the outcomes between journals could be comparable. So I created a scientific version of Mad Libs.

The paper took this form: Molecule X from lichen species Y inhibits the growth of cancer cell Z. To substitute for those variables, I created a database of molecules, lichens, and cancer cell lines and wrote a computer program to generate hundreds of unique papers. Other than those differences, the scientific content of each paper is identical.

The fictitious authors are affiliated with fictitious African institutions. I generated the authors, such as Ocorrafoo M. L. Cobange, by randomly permuting African first and last names harvested from online databases, and then randomly adding middle initials. For the affiliations, such as the Wasse Institute of Medicine, I randomly combined Swahili words and African names with generic institutional words and African capital cities. My hope was that using developing world authors and institutions would arouse less suspicion if a curious editor were to find nothing about them on the Internet.

The papers describe a simple test of whether cancer cells grow more slowly in a test tube when treated with increasing concentrations of a molecule. In a second experiment, the cells were also treated with increasing doses of radiation to simulate cancer radio-

therapy. The data are the same across papers, and so are the conclusions: The molecule is a powerful inhibitor of cancer cell growth, and it increases the sensitivity of cancer cells to radiotherapy.

There are numerous red flags in the papers, with the most obvious in the first data plot. The graph’s caption claims that it shows a “dose-dependent” effect on cell growth—the paper’s linchpin result—but the data clearly show the opposite. The molecule is tested across a staggering five orders of magnitude of concentrations, all the way down to picomolar levels. And yet, the effect on the cells is modest and identical at every concentration.

One glance at the paper’s Materials & Methods section reveals the obvious explanation for this outlandish result. The molecule was dissolved in a buffer containing an unusually large amount of



ethanol. The control group of cells should have been treated with the same buffer, but they were not. Thus, the molecule's observed "effect" on cell growth is nothing more than the well-known cytotoxic effect of alcohol.

The second experiment is more outrageous. The control cells were not exposed to any radiation at all. So the observed "interactive effect" is nothing more than the standard inhibition of cell growth by radiation. Indeed, it would be impossible to conclude anything from this experiment.

To ensure that the papers were both fatally flawed and credible submissions, two independent groups of molecular biologists at Harvard University volunteered to be virtual peer reviewers. Their first reaction, based on their experience reviewing papers

from developing world authors, was that my native English might raise suspicions. So I translated the paper into French with Google Translate, and then translated the result back into English. After correcting the worst mistranslations, the result was a grammatically correct paper with the idiom of a non-native speaker.

The researchers also helped me fine-tune the scientific flaws so that they were both obvious and "boringly bad." For example, in early drafts, the data were so unexplainably weird that they became "interesting"—perhaps suggesting the glimmer of a scientific breakthrough. I dialed those down to the sort of common blunders that a peer reviewer should easily interdict.

The paper's final statement should chill any reviewer who reads that far. "In the next step, we will prove that molecule X is effective



CREDIT: DAVID QUINN AND DANIEL WIESMANN



against cancer in animal and human. We conclude that molecule X is a promising new drug for the combined-modality treatment of cancer.” If the scientific errors aren’t motivation enough to reject the paper, its apparent advocacy of bypassing clinical trials certainly should be.

The sting

Between January and August of 2013, I submitted papers at a rate of about 10 per week: one paper to a single journal for each publisher. I chose journals that most closely matched the paper’s subject. First choice would be a journal of pharmaceutical science or cancer biology, followed by general medicine, biology, or chemistry. In the beginning, I used several Yahoo e-mail addresses for the submission process, before eventually creating my own e-mail service domain, afra-mail.com, to automate submission.

A handful of publishers required a fee be paid up front for paper submission. I struck them off the target list. The rest use the standard open-access “gold” model: The author pays a fee if the paper is published.

If a journal rejected the paper, that was the end of the line. If a journal sent review comments that asked for changes to layout or format, I complied and resubmitted. If a review addressed any of the paper’s serious scientific problems, I sent the editor a “revised” version that was superficially improved—a few more photos of lichens, fancier formatting, extra details on methodology—but without changing any of the fatal scientific flaws.

After a journal accepted a paper, I sent a standard e-mail to the editor: “Unfortunately, while revising our manuscript we discovered an embarrassing mistake. We see now that there is a serious flaw in our experiment which invalidates the conclusions.” I then withdrew the paper.

The results

By the time *Science* went to press, 157 of the journals had accepted the paper and 98 had rejected it. Of the remaining 49 journals, 29 seem to be derelict: websites abandoned by their creators. Editors from the other 20 had e-mailed the fictitious corresponding authors stating that the paper was still under review; those, too, are excluded from this analysis. Acceptance took 40 days on average, compared to 24 days to elicit a rejection.

Of the 255 papers that underwent the entire editing process to acceptance or rejection, about 60% of the final decisions occurred with no sign of peer review. For rejections, that’s good news: It

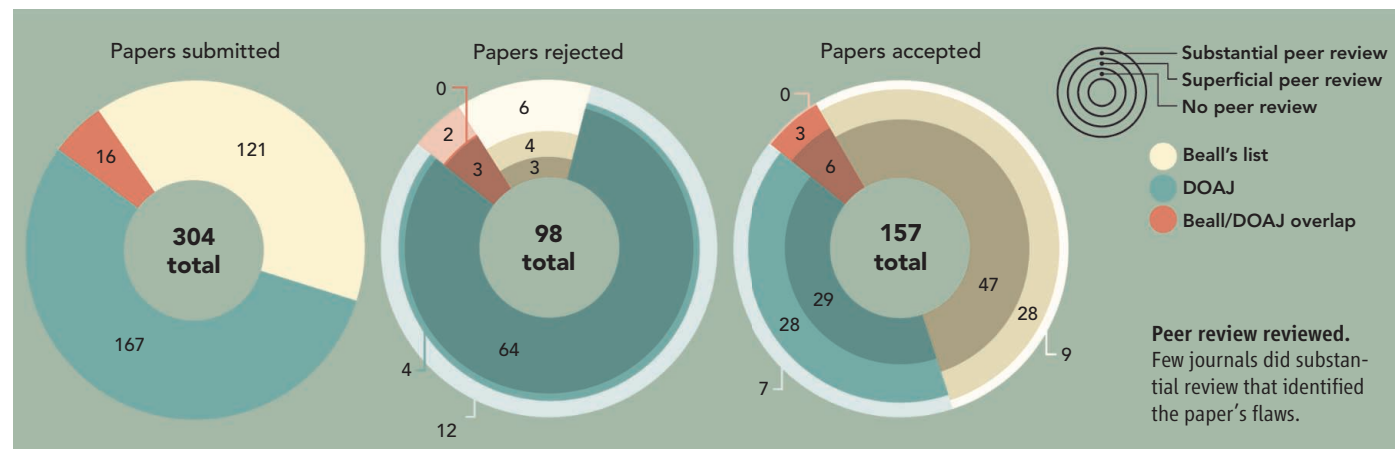
means that the journal’s quality control was high enough that the editor examined the paper and declined it rather than send it out for review. But for acceptances, it likely means that the paper was rubber-stamped without being read by anyone.

Of the 106 journals that discernibly performed any review, 70% ultimately accepted the paper. Most reviews focused exclusively on the paper’s layout, formatting, and language. This sting did not waste the time of many legitimate peer reviewers. Only 36 of the 304 submissions generated review comments recognizing any of the paper’s scientific problems. And 16 of those papers were accepted by the editors despite the damning reviews.

The results show that Beall is good at spotting publishers with poor quality control: For the publishers on his list that completed the review process, 82% accepted the paper. Of course that also means that almost one in five on his list did the right thing—at least with my submission. A bigger surprise is that for DOAJ publishers that completed the review process, 45% accepted the bogus paper. “I find it hard to believe,” says Bjørnshauge, the DOAJ founder. “We have been working with the community to draft new tighter criteria for inclusion.” Beall, meanwhile, notes that in the year since this sting began, “the number of predatory publishers and predatory journals has continued to escalate at a rapid pace.”

A striking picture emerges from the global distribution of open-access publishers, editors, and bank accounts. Most of the publishing operations cloak their true geographic location. They create journals with names like the *American Journal of Medical and Dental Sciences* or the *European Journal of Chemistry* to imitate—and in some cases, literally clone—those of Western academic publishers. But the locations revealed by IP addresses and bank invoices are continents away: Those two journals are published from Pakistan and Turkey, respectively, and both accepted the paper. The editor-in-chief of the *European Journal of Chemistry*, Hakan Arslan, a professor of chemistry at Mersin University in Turkey, does not see this as a failure of peer review but rather a breakdown in trust. When a paper is submitted, he writes in an e-mail, “We believe that your article is original and [all of] your supplied information is correct.” The *American Journal of Medical and Dental Sciences* did not respond to e-mails.

About one-third of the journals targeted in this sting are based in India—overtly or as revealed by the location of editors and bank accounts—making it the world’s largest base for open-access publishing; and among the India-based journals in my sample, 64 accepted the fatally flawed papers and only 15 rejected it.



The United States is the next largest base, with 29 acceptances and 26 rejections. (Explore a global wiring diagram of open-access publishing at <http://scim.ag/OA-Sting>.)

But even when editors and bank accounts are in the developing world, the company that ultimately reaps the profits may be based in the United States or Europe. In some cases, academic publishing powerhouses sit at the top of the chain.

Journals published by Elsevier, Wolters Kluwer, and Sage all accepted my bogus paper. Wolters Kluwer Health, the division responsible for the Medknow journals, “is committed to rigorous adherence to the peer-review processes and policies that comply with the latest recommendations of the International Committee of Medical Journal Editors and the World Association of Medical Editors,” a Wolters Kluwer representative states in an e-mail. “We have taken immediate action and closed down the *Journal of Natural Pharmaceuticals*.”

In 2012, Sage was named the Independent Publishers Guild Academic and Professional Publisher of the Year. The Sage publication that accepted my bogus paper is the *Journal of International Medical Research*. Without asking for any changes to the paper’s scientific content, the journal sent an acceptance letter and an invoice for \$3100. “I take full responsibility for the fact that this spoof paper slipped through the editing process,” writes Editor-in-Chief Malcolm Lader, a professor of psychopharmacology at King’s College London and a fellow of the Royal Society of Psychiatrists, in an e-mail. He notes, however, that acceptance would not have guaranteed publication: “The publishers requested payment because the second phase, the technical editing, is detailed and expensive. ... Papers can still be rejected at this stage if inconsistencies are not clarified to the satisfaction of the journal.” Lader argues that this sting has a broader, detrimental effect as well. “An element of trust must necessarily exist in research including that carried out in disadvantaged countries,” he writes. “Your activities here detract from that trust.”

The Elsevier journal that accepted the paper, *Drug Invention Today*, is not actually owned by Elsevier, says Tom Reller, vice president for Elsevier global corporate relations: “We publish it for someone else.” In an e-mail to *Science*, the person listed on the journal’s website as editor-in-chief, Raghavendra Kulkarni, a professor of pharmacy at the BLDEA College of Pharmacy in Bijapur, India, stated that he has “not had access to [the] editorial process by Elsevier” since April, when the journal’s owner “started working on [the] editorial process.” “We apply a set of criteria to all journals before they are hosted on the Elsevier platform,” Reller says. As a result of the sting, he says, “we will conduct another review.”

The editor-in-chief of the *Kobe Journal of Medical Sciences*, Shun-ichi Nakamura, a professor of medicine at Kobe University in Japan, did not respond to e-mails. But his assistant, Reiko Kharbas, writes that “Upon receiving the letter of acceptance, Dr. Obalanefah withdrew the paper,” referring to the standard final e-mail I sent to journals that accepted the paper. “Therefore, the letter of acceptance we have sent ... has no effect whatsoever.”

Other publishers are glad to have dodged the bullet. “It is a relief to know that our system is working,” says Paul Peters, chief

strategy officer of Hindawi, an open-access publisher in Cairo. Hindawi is an enormous operation: a 1000-strong editorial staff handling more than 25,000 articles per year from 559 journals. When Hindawi began expanding into open-access publishing in 2004, Peters admits, “we looked amateurish.” But since then, he says, “publication ethics” has been their mantra. Peer reviewers at one Hindawi journal, *Chemotherapy Research and Practice*, rejected my paper after identifying its glaring faults. An editor recommended I try another Hindawi journal, *ISRN Oncology*; it, too, rejected my submission.

Coda

From the start of this sting, I have conferred with a small group of scientists who care deeply about open access. Some say that the open-access model itself is not to blame for the poor quality control revealed by *Science*’s investigation. If I had targeted traditional, subscription-based journals, Roos told me, “I strongly suspect you would get the same result.” But open access has multiplied that underclass of journals, and the number of papers they publish.

“Everyone agrees that open-access is a good thing,” Roos says. “The question is how to achieve it.”

The most basic obligation of a scientific journal is to perform peer review, arXiv founder Ginsparg says. He laments that a large proportion of open-access scientific publishers “clearly are not doing that.” Ensuring that journals honor their obligation is a challenge that the scientific community must rise to. “Journals without quality control are

destructive, especially for developing world countries where governments and universities are filling up with people with bogus scientific credentials,” Ginsparg says.

As for the publisher that got Aline Noutcha to pony up a publication fee, the IP addresses in the e-mails from Scientific & Academic Publishing reveal that the operation is based in China, and the invoice they sent me asked for a direct transfer of \$200 to a Hong Kong bank account.

The invoice arrived with good news: After a science-free review process, one of their journals—the *International Journal of Cancer and Tumor*—accepted the paper. Posing as lead author Alimo Atoa, I requested that it be withdrawn. I received a final message that reads like a surreal love letter from one fictional character to another:

Dear Alimo Atoa,

We fully respect your choice and withdraw your article.

If you are ready to publish your paper, please let me know and I will be at your service at any time.

Sincerely yours,
Grace Groovy

—JOHN BOHANNON

Everyone agrees that
open access is a good
thing. The question is
how to achieve it.

—DAVID ROOS,
UNIVERSITY OF PENNSYLVANIA



The Seer of Science Publishing

Vitek Tracz was ahead of the pack on open access. Now he wants to rewrite the rules of peer review

LONDON—“Nobody reads journals,” says science publisher Vitek Tracz, who has made a fortune from journals. “People read papers.” Tracz sees a grim future for what has been the mainstay of scientific communication, the peer-reviewed print journal. Within the next 10 years, he says, it will cease to exist.

This prophecy ought to carry weight. Over the past 3 decades, Tracz, chairman of a conglomerate called the Science Navigation Group, has helped transform the world of science publishing. His most notable creation to date may be BioMed Central, the first for-

Tracz “always has many irons in the fire; he likes to experiment. That’s unlike the rest of science publishers who are quite conservative and work on standardizing, consolidating, and reducing costs,” says Matthew Cockerill, managing director of BioMed Central, which Tracz sold in 2008. By contrast, he says, “Vitek doesn’t believe in business plans, but in ideas.”

Now, the revolutionary, who calls himself “shy” and “un-neat,” is stirring up what could become one of the biggest controversies yet in scientific publishing. Tracz is setting out to shake the very foundations of contemporary science by abolishing anonymous peer review.



Michelin Guide of science

Tracz was born in 1940 in a Polish village then occupied by the Soviet Union, and soon afterward his family joined relatives in Siberia, where his father worked in a mine. After the war they made it back to Poland, where Tracz, as an undergraduate at the University of Warsaw, tried his hand at architecture for a year and then switched to mathematics. Before he completed his degree, Tracz’s family emigrated to Israel, where he continued his math studies. A year later, he moved to London and studied cinematography at the Slade School of Art. He put down roots and launched Medi-Cine, a company that made educational films for medical doctors. His enthusiasm for filmmaking soon waned, however. Tracz sold Medi-Cine and started up Gower Medical Publishing, which printed full-color medical atlases (at a time when most textbooks were in black and white) and assembled slide collections for lecturers.

Tracz grew bored of textbooks, too. In the early 1980s, he saw an opportunity to create something truly novel. That was the *Current Opinion* journals, publications that offer comprehensive reviews in biology and medicine. Tracz likens them to “Michelin Guides”: “There is a problem with the quantity of literature, just like with the quantity of restaurants available out there. You need some [expert] advice and selection, especially when you’re outside your territory,” he says. He later sold *Current Opinion*’s biology journals to Elsevier, and its clinical journals to Rapid Communications of Oxford, which became part of Thomson.

profit open-access publisher. The pioneering site, founded in 2000 in London, has grown into an empire with more than 250 biology and medicine journals in its stable.

BioMed Central earned Tracz a reputation as a visionary. “He’s one of the most important publishers of the last decade,” says Michael Eisen, a biologist at the University of California, Berkeley, and co-founder of the Public Library of Science (PLOS), a nonprofit open-access publisher that launched its first journal in 2003.

Tracz was quick to grasp how the rise of the Internet in the 1990s could transform scientific communication. In 1996, he launched BioMedNet, an online club for biomedical researchers that included a library of scientific papers and a news service called HMS Beagle, named after the ship that Charles Darwin sailed on to South America. “We had a community of 1 million scientists, biologists, and doctors. It was incredibly popular,” Tracz recalls. Two years later, at the height of BioMedNet’s popularity, Tracz sold the site for an undisclosed sum to publishing giant Elsevier, which closed the site in 2004.

Unleashing a juggernaut

As Tracz formed and spun off companies, the “whirlwind,” as one colleague calls him, understood that scientific publishing, as we knew it before the Web, was doomed. Instead of every library stocking a paper journal, a single freely accessible copy of an article or journal was “enough for the whole world,” Tracz says. “The monopolistic power of the publisher suddenly disappeared. So we started thinking: ‘What does it mean? What can we do?’”

Tracz embraced open-access publishing, a movement that blossomed in the late 1990s among scientists and librarians who were angered by the high cost of journal subscriptions at a time when the Web was in principle lowering production and distribution costs. In that ferment, Tracz launched BioMed Central. “Our task was to demonstrate the [system’s] viability for commercial publishers to take it on and switch their model,” Tracz says. After dismissing advertising as a revenue source, the BioMed Central team hit upon the idea that has driven the growth of open-access publishing ever since: making up for lost subscription income by charging authors—or their funders—a publishing fee.

It was a “shrewd” move, says Derk Haank, CEO of Springer Science+Business Media in Berlin. “Vitek made the very wise decision to harness the energy of a ‘movement’ and build a healthy, sustainable business based on it.” In a sign of Biomed Central’s robust health, Springer bought the venture from Tracz in 2008 for an undisclosed sum.

Tracz not only made a business of open access; he also proselytized on its merits. He served on the board of PubMed Central, the U.S. National Institutes of Health’s (NIH’s) online repository of biology and medicine articles, gaining a platform from which he pushed governments and research funders to mandate and fund open-access publishing.

With the open-access juggernaut gaining momentum, Tracz turned his attention to the “other big problems” in science publishing, starting with what he calls the “impact factor poison.” Impact factor measures the average number of citations for each paper published by a journal, and is used as a measure of a journal’s prestige. Critics like Tracz note that impact factor doesn’t reflect the quality of individual papers and, as such, gives little guidance to scientists—or to those making funding or recruitment decisions.

“The impact factor is both problematic and idiotic,” Tracz says. PubMed’s user research, he says, indicates that scientists search for papers that answer their questions, no matter which journal they are published in.

As an alternative metric, Tracz in 2002 launched Faculty of 1000, a directory of biology articles selected, rated, and commented upon by a handpicked group of 5000 experts. This summer, Tracz’s Science Navigation Group started a similar venture, *F1000Trials*, for clinical research.

Scientists say that they use the site, which has since added medicine articles and changed its name to *F1000Prime*, to discover important papers outside their field of expertise, although the site’s metrics for flagging and scoring articles have not caught on widely. But that may be about to change: In August, PLOS added *F1000Prime* data and scores to its article-level metrics—

the citation data, social media usage, and comments that serve as indicators of quality and impact.

A heretical idea

In another bold strike, Tracz is taking aim at science’s life force: peer review. “Peer review is sick and collapsing under its own weight,” he contends. The biggest problem, he says, is the anonymity granted to reviewers, who are often competing fiercely for priority with authors they are reviewing. “What would be their reason to do it quickly?” Tracz asks. “Why would they not steal” ideas or data?

Anonymous review, Tracz notes, is the primary reason why months pass between submission and publication of findings.

“Delayed publishing is criminal; it’s nonsensical,” he says. “It’s an artifact from an irrational, almost religious belief” in the peer-review system.

As an antidote, the heretic in January launched a new venture that has dispensed altogether with anonymous peer review: *F1000Research*, an online outlet for immediate scholarly publishing. “As soon as we receive a paper, we

publish it,” after a cursory quality check. Peer review happens *after* publication, and in the light of day. *F1000Research* selects referees, who post their names and affiliations alongside their critiques. Papers become like wikis, with reviewers and authors posting comments and revisions as the need arises.

F1000Research requires authors to submit the full data set underlying a paper—not just selected graphs or analyses. Readers “don’t just want the narrative of what you think you found, but what you actually found,” Tracz says. What authors get in return, he says, is ownership of data from the moment of publication. The price of publishing in a traditional journal now could be steep, Tracz argues, as scientists could lose priority for a discovery. He also sees a role for *F1000Research* in publishing orphan studies: negative findings (see p. 68) and incremental advances that most journals ignore.

“When Vitek told me about it at the beginning, I told him it’s kind of crazy,” says David Lipman, director of the NIH’s National Center for Biotechnology Information, which is home to PubMed Central. But Lipman says that he is starting to come around as *F1000Research* takes shape. “You can see examples on the site of perfectly solid articles,” says Lipman, who now calls it “a very attractive option” for researchers. Critics, however, have questioned the reliability of publishing before peer review. On The Scholarly Kitchen blog, Kent Anderson, a former executive at *The New England Journal of Medicine*, described *F1000Research*’s publishing model as “surreal” and “topsy-turvy.”

Tracz acknowledges that in reshaping peer review, he’s taking on a sacred cow. “There will be some growing pains,” he says. But his maverick ideas tend to become mainstream over time. “At the beginning of open access,” one colleague says, Tracz “was ridiculed by other [publishers].” No one ridicules open access now.

“He’s not radical,” Eisen insists, “just sensible. Sensible doesn’t [usually] happen in scientific publishing.” The coming years will see whether open peer review is sensible—or too radical for most researchers to stomach.

—TANIA RABESANDRATANA

Peer review is sick and collapsing under its own weight.

—VITEK TRACZ,
SCIENCE NAVIGATION GROUP

He’s not radical, just sensible.

—MICHAEL EISEN,
PUBLIC LIBRARY OF SCIENCE



The Power of Negative Thinking

Gaining ground in the ongoing struggle to coax researchers to share negative results

Glenn Begley was stymied. At the drug giant Amgen, where Begley was vice-president and global head of hematology and oncology research, he was struggling to repeat an animal study of tumor growth by a respected oncologist, published in *Cancer Cell*. One figure stood out as particularly impressive. But it was proving stubbornly resistant to replication.

In March of 2011, Begley saw a chance to play detective. At the annual meeting of the American Association for Cancer Research in Orlando, he and a colleague invited the paper's senior author out to breakfast. Across his orange juice and oatmeal, Begley floated the question: Why couldn't his group get the same finding as the oncologist?

The oncologist, whom Begley declines to name, had an easy explanation. "He said, 'We did this experiment a dozen times, got this answer once, and that's the one we decided to publish.'"

Begley was aghast. "I thought I'd completely misheard him," he says, thinking back on the encounter. "It was disbelief, just disbelief."

As it turned out, the respected oncologist was in good company. A year later, Begley and Lee Ellis, a surgical oncologist at MD Anderson Cancer Center in Houston, published a commentary in *Nature* about lax standards in preclinical research in cancer. They shared that Amgen scientists couldn't replicate 47 of 53 landmark cancer studies in animals, including the respected oncologist's. (Last year, Begley left Amgen to become chief scientific officer for TetraLogic Pharmaceuticals, a small company based in Malvern, Pennsylvania.)

Striking as it is, Begley and Ellis's exposé is part of a pattern. In fields from clinical medicine to psychology, studies are showing that

the literature is filled with papers that present results as stronger than they actually are—and rarely report negative outcomes.

What makes it so difficult to portray negative results straight up? Along with the drive to prove one's theories right, "there is a wide perception that negative studies are useless, indeed a failure," says Douglas Altman, a statistician at the University of Oxford in the United Kingdom. Journals, too, often "like big, exciting, positive findings," he says. As a result, negative results are often relegated to the dustbin, a problem commonly called the "file drawer effect." Or, by cherry-picking data or spinning the conclusions, a paper may make a negative study seem more positive. But those practices are being challenged.

In some fields, "there's increasing recognition" that leaving negative findings unpublished is "not the right thing to do," says David Allison, director of the Nutrition Obesity Research Center at the University

of Alabama, Birmingham, where he studies methodologies in obesity research. Furthermore, as journals move online, where pages are limitless, and as more pledge to treat positive and negative studies equally in the review process, the venues for publishing null findings are expanding.

Still, many researchers and journals continue to cast results as a story that they believe others will want to read. They choose their words carefully, describing, say, an obesity increase as "alarming" rather than "modest," Allison suggests. Or investigators dig through mounds of data in the hunt for shiny nuggets. "Is there some gold in these hills? ... We know if you sift through the data enough," Allison says, "you'll find things," even if by chance.

A matter of emphasis

Many who study negative results have focused on clinical trials, in part because biased results can directly affect patients, and because a published trial can often be compared with the original goals and protocol, which these days is often publicly available. That makes it easier to see whether a published study accentuates the positive.

Altman and clinical epidemiologist Isabelle Boutron, working together at Oxford about 5 years ago, set out to explore these effects. They and their colleagues culled 616 randomized clinical trials from a public database, all of them published in December 2006, and focused on 72 whose primary goals hadn't panned out. The group hunted for "spin" in the papers, defining it as the "use of specific reporting strategies, from whatever motive, to highlight that the experimental treatment is beneficial, despite a statistically non-significant difference for the primary outcome."

In 13 papers, the title contained spin, as did 49 abstracts and 21 results sections, Altman and Boutron reported in 2010 in the *Journal of the American Medical Association*. Some authors compared patients before and after treatment, instead of comparing those who got the experimental drug and those who didn't. In one case, authors contrasted the treated patients not with their comparison group in the same trial, but with a placebo group from another study to argue that the treatment was superior.

Boutron says the authors of these papers were upfront about the fact that their study's original goal hadn't been met. Still, "often they tried to spin" what had happened, says Boutron, now at Université Paris Descartes.

She acknowledges that in clinical research, negative results can be difficult to interpret. If a well-designed trial finds that a new drug eases symptoms, it probably does. But finding the opposite doesn't necessarily mean the therapy is hopeless. When a therapy's superiority isn't statistically significant, it might still help—or the difference could be due to chance. "You won't be able to provide a black-and-white answer," says Boutron, who is now struggling to write up a negative trial herself.

It's not just clinical research. So-called "soft" sciences, such as psychology, carry an even greater risk of biased reporting, according to Daniele Fanelli, who studies bias and misconduct at the University of Montreal in Canada. In August, Fanelli and epidemiologist John Ioannidis at Stanford University added an intriguing twist: They found behavioral research was more likely to claim positive results if the corresponding author was based in the United States.

To probe why researchers spin negative studies, Altman, medical statistician Paula Williamson at the University of Liverpool, and others decided to ask them. They identified 268 clinical trials in which there were suspicions of selective reporting. Investigators from 59 agreed to be interviewed.

The researchers offered diverse explanations for why they failed to report data on an outcome they'd previously pledged to examine. "It was just uninteresting and we thought it confusing so we left it out," said one in the paper by Williamson's group, published in 2011 in *BMJ*. Another said, "When I take a look at the data I see what best advances the story, and if you include too much data the reader doesn't get the actual important message."

At the *American Journal of Clinical Nutrition* (*AJCN*), Editor-in-Chief Dennis Bier encounters this routinely. A study may be designed to measure whether an intervention will lead to weight loss, say, but the manuscript focuses on blood pressure or cholesterol—originally identified as "secondary endpoints" but now taking on a starring role. The weight loss outcome "may occur in one line in table 3," Bier says. For the most part, "these are not people trying to be duplicitous," he says. "They're trying to get as much as they can out of their data." Still, he says that he and his fellow editors "spend an awful lot of time trying to get things out of papers," and "getting the caveats in."

Scientists rarely admit that these practices result in biased reports. Several whose work was cited in studies of bias did not respond to *Science*'s requests for comment, or insisted that their work was statistically sound. After examining papers positing that reducing sugary drinks consumption decreases obesity, Allison, who takes funding from the soda industry, flagged several papers for "distortion of information." The senior author of one, Barry Popkin of the Univer-

sity of North Carolina, Chapel Hill, School of Public Health, agreed that the abstract overstated the case, suggesting that swapping water or diet drinks for calorie-heavy beverages caused weight loss when that outcome was not statistically significant. Popkin blamed the journal, *AJCN*, for the sentence. "The journal process is an imperfect process," he told *Science*. "That's what the editors wanted to put in there."

Bier agreed that the change had been urged by the journal—but only because, he said, the original version overstated the result to an even greater degree.

Telling it like it is

Forest ecologist Sean Thomas of the University of Toronto in Canada and his Ph.D. student spent 2 years traveling to and from the rainforests of Dominica testing whether, as long suspected, trees need more light the larger they grow. His results were lackluster, but not his publication strategy.

In the end, the relationship between light requirements and tree growth was flimsy, Thomas found, but as with many negative results, he couldn't say for sure that the thesis was wrong. The Ph.D. student, despairing, switched topics. "But I was bound and determined to publish," Thomas says.

"We thought about ways of positively spinning things," he confesses. Then he hit on another solution: submitting the paper to the *Journal of Negative Results: Ecology & Evolutionary Biology*. A group of postdocs at the University of Helsinki conceived of the journal in the early 2000s and launched it in 2004. Its publication record is paltry; it gets only two or three submissions a year. "It could hardly get any lower unless they were publishing nothing," Thomas says.

Undeterred, he sent his paper in, and it appeared in 2011. "We didn't have to package things in a way that might pull out some kind of marginal result," Thomas says. "This was an honest way of presenting the information that would also make it clear what the story was from the beginning."

Are studies that tell it like it is the exception to the rule, or the cusp of a new trend? Most agree that journals for negative results—another exists for biomedical studies—are not a comprehensive solution. Some mainstream journals are getting in on the act: A study presented last month at the International Congress on Peer Review and Biomedical Publication examined how eight medical journals had handled recently submitted papers of clinical trials and found they were just as likely to publish those with negative results as positive ones.

Allison believes that making all raw data public could minimize bias in reporting, as authors face the fact that others will parse their primary work. A more lasting solution may come from a shift in norms: embracing and sharing null findings without regret or shame. "The only way out of this," Fanelli says, is that "people report their studies saying exactly what they did."

Openness has its rewards. Although Thomas's Ph.D. student, Adam Martin, adopted a new thesis topic after the Dominican rainforest flop, as Martin dives into the job market Thomas is urging him to launch his job talk with that story. "It's an unusual enough thing," Thomas says, "that you'll stand out" in a crowded field.

—JENNIFER COUZIN-FRANKEL

The only way out of this [is that] people report their studies saying exactly what they did.

—DANIELE FANELLI,
UNIVERSITY OF MONTREAL



Hey, You've Got to Hide Your Work Away

Debate is simmering over how and when to publish sensitive data

Not long after letters laced with anthrax spores killed five Americans in September 2001, a research team led by genome scientist Harold “Skip” Garner came up with an idea for probing such crimes. But the solution gave him pause. During a study that used new gene technologies to analyze several of the world’s deadliest pathogens, the researchers realized that a unique genetic “barcode” could be discreetly inserted into laboratory strains, potentially enabling forensic scientists to track a bioweapon or escaped pathogen back to its source. It was just the kind of tagging that could have helped investigators identify the source of the weaponized anthrax spores tucked into the deadly letters, says Garner, now with the Virginia Bioinformatics Institute at the Virginia Polytechnic Institute and State University in Blacksburg. But publishing the trick might also aid evil-doers, he realized. “It was information that might be misused, to figure out how to evade detection,” Garner recalled recently. “We had to ask: ‘Is it wise to widely share this?’”

That question is confronting many scientists these days. Every research field has findings so sensitive that scientists can spend countless hours fretting over when, where, and how to publish them—or whether to share them at all. For microbiologists and chemists, it might be a technique that could be misused to create a terrifying weapon. For biomedical and social scientists, huge databases of personal health

and behavioral information pose threats to privacy. Archaeologists and wildlife biologists worry about pinpointing some study sites, fearful they could guide looters and poachers to priceless artifacts or vulnerable species. It’s not a new conundrum. Academic researchers have long struggled to balance an ethos of openness with demands for secrecy. Most famously, perhaps, U.S. nuclear scientists in the late 1930s and early 1940s kept mum about findings that they worried might give Nazi Germany clues to building an atom bomb. Today’s struggles over sensitive data may have lower stakes, but they are increasingly pervasive and complex. More scientists are engaged in work that requires secrecy in the name of protecting national security, intellectual property, and confidentiality. And researchers may not get to make the call; government regulators, corporate lawyers, and even ethicists are demanding a bigger say in deciding when to let information loose and when to lock it up. Ironically, some agencies publish classified journals for scientists with security clearances to have a place to share peer-reviewed secrets (see sidebar, p. 71).

Rude awakening

Such trends are forcing scientists to rethink how they publish papers and agencies to reconsider which studies to fund. And they are helping spur the growth of new bureaucracies designed to avoid the unintentional release of sensitive data—or even prevent such data’s creation in the first place. Whether such controls will ultimately help or harm science and society, however, is the subject of vigorous debate. “Open communication among scientists is critical to the good that science can do, but concerns about the misuse of information are testing that ideal,” says ethicist and molecular biologist Kathleen Kolakovich Eggleston of the University of Notre Dame in Indiana. And with the Internet making it nearly impossible to recapture sensitive data once they have escaped, she says, “it’s an issue that’s just going to become even more problematic.”

could escape from a lab and cause a human pandemic, or that revealing details about the research could inadvertently aid terrorists seeking a bioweapon. They asked the National Science Advisory Board for Biosecurity, set up after the 2001 anthrax attacks, to review the H5N1 studies. It initially recommended that the papers be published only if journal editors deleted key methodological details and shared them only with “responsible” scientists. In the end, however, such selective censorship proved both practically and legally impossible, and a divided advisory board ultimately supported full publication of both studies, which appeared in print last year.

The episode has left a mark on science. For instance, it prompted the government of the Netherlands to take the unusual step of requiring researchers there to obtain an export permit before sending their final manuscript to *Science* in the United States—a precedent for government oversight of data sharing that worries some scientists. And it prompted the U.S. National Institutes of Health (NIH), the world’s biggest biomedical science funder, to impose extensive new rules. NIH-funded researchers and universities now must undertake special reviews of proposed studies that involve H5N1 and more than a dozen other risky biological agents and toxins. The goal: to identify experiments that might produce sensitive “dual use” findings that could be used for good and evil—and force alterations or even stop them before they begin. If NIH were to fund a study that meets the dual use definition, the agency announced in August, researchers must create “risk mitigation” plans that include strategies to control sharing sensitive results. And they must allow NIH to see manuscripts and abstracts at least 10 days prior to submission to a journal or meeting.

Such prior review requirements are already common in academic studies funded by industry, which is keen to patent profitable ideas before they become public, and military agencies, which aren’t eager to aid adversaries. Still, some researchers fear that NIH’s adoption of prior review for a new swath of academic science could signal a creeping expansion of bureaucratic controls. Journal editors and legal specialists say it’s not clear whether the U.S.

government can legally block publication of NIH-funded data unless it takes the radical step of classifying them as secret.

Such questions may not be resolved for some time. In the short term, the new rules are expected to affect just a handful of studies. Editors of major scientific journals say that they rarely see truly sensitive manuscripts. A 2008 study, for instance, found that just six of 16,000 manuscripts submitted to the 11 journals published by the American Society for Microbiology over a 5-year period raised dual use concerns. Just two weren’t published because the authors wanted to withhold methodological details.

A dearth of worrisome manuscripts doesn’t mean people aren’t making worrisome discoveries; researchers may simply be sitting on sensitive results. In a paper to be published later this year by the *Saint Louis University Journal of Health Law & Policy*, David

Franz, former commander of the U.S. Army Medical Research Institute of Infectious Diseases in Frederick, Maryland, recalls that, in the 1990s, scientists there unintentionally created a virus strain that was resistant to a potential treatment. After a discussion, “we decided to put the entire experiment into the autoclave,” Franz tells *Science*. “That was it. We didn’t hear anyone say: ‘Wow, we could get a paper in *Science* or *Nature*.’”

Garner took a similarly cautious approach with his barcoding technology. “We wrote up a white paper for some of the government agencies, but didn’t distribute it widely,” he says. “Seemed better that way.”

Censorship or discretion?

In other fields, scientists are learning that they may give away sensitive data without being aware they’d let it slip. Archaeologists have posted pictures of new finds on websites only to discover that savvy thieves have tapped metadata digitally attached to images to discover location information—and then looted the site. Conservation biolo-

Cloak-and-Dagger Publishing

A hot new journal debuted last month, but you can’t read it—or publish in it—unless you have a security clearance from the U.S. government. The *Journal of Sensitive Cyber Research and Engineering* (JSCoRE) is the newest addition to the shadowy shelf of “dark,” or classified, journals that aim to solve a thorny problem: how to rigorously peer review and share sensitive government-funded findings that officials don’t want sent to regular journals.

“Even though the community of researchers doing sensitive work has the same needs as those doing unrestricted research, the absence of a peer-reviewed publication ... impedes the quality and progression of sensitive science,” wrote JSCoRE co-editor William “Brad” Martin of the U.S. National Security Agency and colleagues in a poster on the journal’s origins that they presented at a meeting last year. To help researchers in the booming field leap that

obstacle, the poster promises that JSCoRE will “feature an editorial board consisting of cyber luminaries from inside and outside of government” and “qualified peer reviewers.”

JSCoRE may reside where few can lay eyes on it, but it has plenty of company. Worldwide, intelligence services and military forces have long published secret journals that often touch on technical topics. The demand for restricted outlets is bound to grow as governments classify more information; the United States alone has dozens of categories of controlled information, including “top secret,” “for official use only,” and “sensitive but unclassified.” But going dark doesn’t mean keeping the general public entirely in the dark: JSCoRE has asked authors to provide titles and abstracts that don’t have to be kept secret, so the journal can appear in public indexes.

—D. M.

gists often refrain from saying exactly where they’ve spotted a rare species, for fear an overzealous collector or landowner will hunt it down. Genome researchers and social scientists have been stung by computer wizards who have shown that they can take databases that supposedly have been stripped of information allowing the identification of individuals and “re-identify” study participants, violating privacy rules. In theory, such techniques could reveal a trove of problematic information, such as embarrassing Web surfing habits, stigmatizing mental health issues, or genetic traits that could affect employment or insurance.

As plant biologist Rodrigo Gutiérrez of the Catholic University of Chile in Santiago puts it: “We are gaining the capacity to generate lots of sensitive information, but not necessarily the capacity to handle it appropriately.”

—DAVID MALAKOFF

could escape from a lab and cause a human pandemic, or that revealing details about the research could inadvertently aid terrorists seeking a bioweapon. They asked the National Science Advisory Board for Biosecurity, set up after the 2001 anthrax attacks, to review the H5N1 studies. It initially recommended that the papers be published only if journal editors deleted key methodological details and shared them only with “responsible” scientists. In the end, however, such selective censorship proved both practically and legally impossible, and a divided advisory board ultimately supported full publication of both studies, which appeared in print last year.

The episode has left a mark on science. For instance, it prompted the government of the Netherlands to take the unusual step of requiring researchers there to obtain an export permit before sending their final manuscript to *Science* in the United States—a precedent for government oversight of data sharing that worries some scientists. And it prompted the U.S. National Institutes of Health (NIH), the world’s biggest biomedical science funder, to impose extensive new rules. NIH-funded researchers and universities now must undertake special reviews of proposed studies that involve H5N1 and more than a dozen other risky biological agents and toxins. The goal: to identify experiments that might produce sensitive “dual use” findings that could be used for good and evil—and force alterations or even stop them before they begin. If NIH were to fund a study that meets the dual use definition, the agency announced in August, researchers must create “risk mitigation” plans that include strategies to control sharing sensitive results. And they must allow NIH to see manuscripts and abstracts at least 10 days prior to submission to a journal or meeting.

Such prior review requirements are already common in academic studies funded by industry, which is keen to patent profitable ideas before they become public, and military agencies, which aren’t eager to aid adversaries. Still, some researchers fear that NIH’s adoption of prior review for a new swath of academic science could signal a creeping expansion of bureaucratic controls. Journal editors and legal specialists say it’s not clear whether the U.S.

government can legally block publication of NIH-funded data unless it takes the radical step of classifying them as secret.

Such questions may not be resolved for some time. In the short term, the new rules are expected to affect just a handful of studies. Editors of major scientific journals say that they rarely see truly sensitive manuscripts. A 2008 study, for instance, found that just six of 16,000 manuscripts submitted to the 11 journals published by the American Society for Microbiology over a 5-year period raised dual use concerns. Just two weren’t published because the authors wanted to withhold methodological details.

A dearth of worrisome manuscripts doesn’t mean people aren’t making worrisome discoveries; researchers may simply be sitting on sensitive results. In a paper to be published later this year by the *Saint Louis University Journal of Health Law & Policy*, David

Franz, former commander of the U.S. Army Medical Research Institute of Infectious Diseases in Frederick, Maryland, recalls that, in the 1990s, scientists there unintentionally created a virus strain that was resistant to a potential treatment. After a discussion, “we decided to put the entire experiment into the autoclave,” Franz tells *Science*. “That was it. We didn’t hear anyone say: ‘Wow, we could get a paper in *Science* or *Nature*.’”

Garner took a similarly cautious approach with his barcoding technology. “We wrote up a white paper for some of the government agencies, but didn’t distribute it widely,” he says. “Seemed better that way.”

Censorship or discretion?

In other fields, scientists are learning that they may give away sensitive data without being aware they’d let it slip. Archaeologists have posted pictures of new finds on websites only to discover that savvy thieves have tapped metadata digitally attached to images to discover location information—and then looted the site. Conservation biolo-

Cloak-and-Dagger Publishing

A hot new journal debuted last month, but you can’t read it—or publish in it—unless you have a security clearance from the U.S. government. The *Journal of Sensitive Cyber Research and Engineering* (JSCoRE) is the newest addition to the shadowy shelf of “dark,” or classified, journals that aim to solve a thorny problem: how to rigorously peer review and share sensitive government-funded findings that officials don’t want sent to regular journals.

“Even though the community of researchers doing sensitive work has the same needs as those doing unrestricted research, the absence of a peer-reviewed publication ... impedes the quality and progression of sensitive science,” wrote JSCoRE co-editor William “Brad” Martin of the U.S. National Security Agency and colleagues in a poster on the journal’s origins that they presented at a meeting last year. To help researchers in the booming field leap that

obstacle, the poster promises that JSCoRE will “feature an editorial board consisting of cyber luminaries from inside and outside of government” and “qualified peer reviewers.”

JSCoRE may reside where few can lay eyes on it, but it has plenty of company. Worldwide, intelligence services and military forces have long published secret journals that often touch on technical topics. The demand for restricted outlets is bound to grow as governments classify more information; the United States alone has dozens of categories of controlled information, including “top secret,” “for official use only,” and “sensitive but unclassified.” But going dark doesn’t mean keeping the general public entirely in the dark: JSCoRE has asked authors to provide titles and abstracts that don’t have to be kept secret, so the journal can appear in public indexes.

—D. M.

gists often refrain from saying exactly where they’ve spotted a rare species, for fear an overzealous collector or landowner will hunt it down. Genome researchers and social scientists have been stung by computer wizards who have shown that they can take databases that supposedly have been stripped of information allowing the identification of individuals and “re-identify” study participants, violating privacy rules. In theory, such techniques could reveal a trove of problematic information, such as embarrassing Web surfing habits, stigmatizing mental health issues, or genetic traits that could affect employment or insurance.

As plant biologist Rodrigo Gutiérrez of the Catholic University of Chile in Santiago puts it: “We are gaining the capacity to generate lots of sensitive information, but not necessarily the capacity to handle it appropriately.”

—DAVID MALAKOFF



Lock Up the Genome, Lock Down Research?

Researchers say that gene patents impede data sharing and innovation; patent lawyers say there's no evidence for this

"Your Genes Have Been Freed." declared a website banner posted on 13 June by Ambry Genetics, a small California firm that analyzes DNA. Earlier that day, the U.S. Supreme Court ruled that raw human DNA is not patentable. Ambry cheered the decision because it wiped out some intellectual property claims on genes "owned" by other firms. Now it seemed that anyone could roam the human genome and use any genes—without a license from the owner.

Ambry and another small company—Gene By Gene, based in Houston, Texas—immediately began to offer to test U.S. clients for two gene variants linked to breast and ovarian cancer, *BRCA1* and *BRCA2*. Prior to the court's ruling, those sequences had been the exclusive property of Myriad Genetics in Salt Lake City. Myriad was the first to isolate the genes, won U.S. patents on them in the mid-1990s, and launched and fiercely defended a *BRCA* testing monopoly that charges more than \$3000 per test. Last year alone, Myriad earned close to \$500 million. Myriad's business, however, was built on the view that naturally occurring DNA can be patented. The company lost that argument in a lengthy legal battle—*Association for Molecular Pathology v. Myriad*—that went all the way to the Supreme Court (*Science*, 21 June, p. 1387).

Many academics and clinicians submitted court briefs opposing Myriad, arguing that no company should have so much control over human genetic information. Even Francis Collins, director of the U.S. National Institutes of Health (NIH), said he liked the court's ruling because it would benefit research.

Myriad is already engaged in a fresh court battle in Utah with Ambry and Gene By Gene. But the Myriad ruling has rekindled debate over just how the U.S. patent system—and gene patenting in particular—affects the conduct of science. The combatants agree that, in principle, the U.S. patent system is intended to encourage the free flow of new knowledge so that society can benefit. In exchange for revealing the details of discoveries so that others can build on them, inventors get patents that give them the right to charge fees to users for up to 20 years—and to go to court if they think someone is infringing.

In practice, however, critics say the system can work against innovators. Instead of promoting the sharing of ideas, it is often used to dam up knowledge. A handful of recent studies, for instance, have concluded that gene-related intellectual property has created a legal thicket that stymies biomedical science and locks away data that could improve clinical tests. Similar, but more muted, complaints have emerged in other fields, from computer science to engineering. That's far from the innovation and sharing that the patent system is supposed to encourage, critics add.

On the other side, champions of the patent system, including many lawyers and a former patent court chief judge who spoke with *Science*, say such attacks are unsupported by the evidence. Claims by gene patent critics, they argue, are based on emotion. "The idea that scientific researchers are being sued or threatened with lawsuits [for doing research] is a fiction," says Paul Michel, former chief judge of the U.S. Court of Appeals for the Federal Circuit, the top patent review body

below the Supreme Court. “I don’t know where this myth comes from.”

Some researchers, meanwhile, are working to sidestep patent battles by making sure that gene sequences and other kinds of data are quickly entered into public databases, where they are free to all.

Skeptics and believers

Bio-patent critics include some high-profile advocates. One is Nobel laureate Joseph Stiglitz, an economist at Columbia University. At its heart, the former Clinton administration official wrote in a 14 July *New York Times* editorial blog, the *BRCA* conflict is about whether patients must pay steep fees to access life-saving technologies and clinicians must get licenses to do research. The fees that Myriad charges for its tests are a reward for invention, he noted. But the price isn’t worth it, he argued, because “the two genes would likely have been isolated . . . soon anyway, as part of the global Human Genome Project.” And as part of that publicly funded effort, the sequences would have been entered into a free database.

Stiglitz was retained as an expert by the groups that sued Myriad in the Supreme Court, and he is consulting for Ambry Genetics and Gene By Gene in the ongoing Utah case. (He has donated his fees from these cases to charity.) In a statement filed with the Utah court, Stiglitz argues that DNA patents “impede the dissemination of information.” In general, economists argue that the “transaction costs” of acquiring privately held data—such as signing an agreement to use a patented gene—discourage use. Recent studies that Stiglitz cited examined whether research papers cited proprietary genes less often than those that were “free.” A 2013 study by economist Heidi Williams at the Massachusetts Institute of Technology in Cambridge found that protected DNA was cited 20% to 30% less, and that genes in the private database of the biotech firm Celera Genetics were 20% to 30% less likely to be used in clinical tests than free genes.

Ambry Genetics and Gene By Gene also submitted a statement to the Utah court by geneticist and bioethicist Mildred Cho of Stanford University in Palo Alto, California. She wrote that her own NIH-funded research had concluded that patents on clinical genetic tests “inhibit scientific research.” A 2001 telephone survey of U.S. lab directors working on gene tests, for instance, found that 53% reported deciding not to develop a new clinical genetic test because of a gene patent or license; two-thirds believed that “gene patents resulted in a decreased ability to do research.” Such data have helped persuade Stiglitz that patents and other property claims on genes have done harm by “discouraging further innovation” or even “not allowing any usage of the scientific information at all.”

A case in point, critics say, is Myriad’s refusal to make public data on potentially harmful *BRCA* variations that it has discovered through its exclusive control of DNA used in gene testing. The company argues that U.S. law requires it to protect patient privacy and control how test results are used. Spokesman Ronald Rogers points out that Myriad has collaborated with dozens of “non-commercial, academic” research labs. But it doesn’t put data in public repositories, which he says don’t guarantee privacy or the quality of clinical interpretation.

In contrast, Gene By Gene Chief Scientific Officer David Mittelman says that his company is “a big fan” of making public

the new gene variants it discovers and is ready to launch an initiative promoting this cause, at freemygenes.org.

Defenders of the patent system argue that all the attacks on gene patents add up to a weak indictment. They say that although researchers may perceive otherwise, there’s no direct evidence that intellectual property owners have impeded anyone from doing research. Michel, for instance, says companies rarely sue scientists; one reason is that it would guarantee bad press but be unlikely to win a big settlement.

Still, to clarify matters, Michel and others would like Congress to enact a law saying that a researcher who uses patented material for science—and not for commerce—is protected from infringement lawsuits. Other nations have such “research exemptions,” and U.S. case law has recognized this rule as a practical matter. But Congress has balked at enshrining it in a statute.

Removing fences

While experts debate the effects of patent law, some researchers are taking direct action to liberate genetic data. To prevent patenting or other limitations—as well as improve standards—they’re scooping up any gene variants they can get from clinics and patients and dumping them into a public database. The repository, known as ClinVar, is maintained by NIH’s National Center for Biotechnology Information. In time, leaders say that they should be able to compile a list of all known human gene variants (such as those for *BRCA1* and *BRCA2*) and their health effects, edited to remove personal information.

Geneticist Heidi Rehm at the Brigham and Women’s Hospital in Boston is a key ClinVar contributor. She heads the Laboratory for Molecular Medicine, which provides gene tests and analysis to clinics in the Partners HealthCare network in Boston, affiliated with Harvard Medical School. The lab has already donated about 7000 variants for 155 genes. In all, 56 groups have signed up to collaborate. But several large gene-testers have not, Rehm says. One of them is Myriad.

“There’s no doubt in my mind that lack of data sharing is harmful to patients,” Rehm says. The lack of a universal data bank of gene variants, for instance, could slow the development of more accurate gene tests. When Rehm’s lab recently worked with two others to see just how well their different genetic tests matched when used on the same genes,

they found “a 20% discrepancy,” she says, suggesting the results “can’t all be right.” Public data could help find and resolve such discrepancies, and ultimately improve health care.

To speed that outcome, the International Collaboration for Clinical Genomics—which includes early ClinVar submitters—met last month at NIH to work out plans for curating information, protecting privacy, and granting database access. NIH has awarded three lead institutions, including Rehm’s, nearly \$25 million over the next 4 years to get the project under way. The aim is to set high standards for data collection and annotation. In addition, it could make some private gene variant collections, like Myriad’s, redundant.

In the meantime, court battles over patented genes continue as judges digest the implications of the Myriad decision. Last month, the Utah court heard arguments on Myriad’s request for an injunction to stop its rivals in California and Texas from offering *BRCA* tests. A decision was pending at press time. It’s not likely to be the last word, and the legal battle could rumble on for months—or years. —ELIOT MARSHALL

[DNA patents]
impede the
dissemination
of information.

—JOSEPH STIGLITZ,
COLUMBIA UNIVERSITY



The Annual Meeting: Improving What Isn't Broken

Annual meetings are moneymakers for most scientific societies, and scientists continue to flock to them. But as the world changes, how long can the status quo hold?

Nearly 22,000 scientists converged on San Francisco last December for a meeting of the American Geophysical Union (AGU). Local hotels and restaurants feasted on the biggest annual gathering of physical scientists on the planet, and AGU turned a tidy profit on what was its largest meeting ever. But in a world in which the main currency of information is now bytes, have such megaconclaves become an endangered species?

There are plenty of reasons to question the future of the traditional annual scientific conference. U.S. agencies have less money to spend on travel, research budgets are tighter, scientists are busier, and Web-based technologies for accessing meetings remotely are improving. But there are few signs that extinction is around the corner.

In fact, the familiar 5-day smorgasbord of talks, poster sessions, exhibition booths, job fairs, and public outreach seems to have

lost none of its appeal for scientists. Meeting attendance has held steady or risen in recent years, according to executives at more than a dozen scientific societies who spoke with *Science*. So, too, have the number of requests to present at meetings, which officials say is a good barometer of overall interest. And compelling presenters continue to pack auditoriums (see p. 78).

At the same time, a one-two budget punch to federal agencies is taking a toll. The first blow was a May 2012 directive from the White House that ordered every agency to cut its spending on travel by 30% from 2010 levels. The cuts, triggered by over-the-top spending by one agency that prompted a public outcry, also come with a \$500,000 cap on the cost of any government-sponsored meeting and closer scrutiny of all travel. The changes have made it much harder for federal scientists to gain permission to attend their favorite conferences.

What's Lost When a Meeting Goes Virtual

Mihály Horányi has been a regular at NASA's annual Lunar Science Forum since its debut in 2008. But when the University of Colorado, Boulder, plasma physicist registered for this summer's conference at NASA's Ames Research Center in Mountain View, California, he didn't bother booking a plane ticket or a hotel room. That's because the meeting had gone virtual.

Horányi, who also directs the Colorado Center for Lunar Dust and Atmospheric Studies, was on the program to describe an instrument that was launched last month aboard a NASA probe to study the moon's dust and thin atmosphere (*Science*, 13 September, p. 1161). But instead of stepping onto a stage in front of hundreds of colleagues, Horányi sat down at his computer at 1:45 p.m. on the first day of the conference and began talking into a webcam perched above the screen.

"Last year it was a performance," he says about an invited talk he gave at the July 2012 forum. "This year it meant staring at myself, being annoyed that I kept leaning in and out of the picture, and thinking, 'Boy, am I getting old.'"

The switch makes the forum the largest scientific gathering to embrace the new world of cyber meetings, says Greg Schmidt, deputy director of NASA's Solar System Exploration Research Virtual Institute. (That's the new name for the Lunar Science Institute [LSI], which reflects the Obama administration's decision to substitute an asteroid for the moon as a target for human exploration.)

NASA officials decided to go virtual because of budget pressures—most participants in the forum are either NASA employees or scientists on NASA-funded projects. Schmidt doesn't know how much money was saved, although he says that the cost of the additional bandwidth and servers needed to conduct the live streaming was much less than that of hosting a physical event.

Institute officials tried to cushion the shock by preserving the forum's usual format. But instead of welcoming some 500 scientists to the Ames campus, the hosts invited participants to log on each day, from 8:30 a.m. to 3 p.m. Pacific time. In addition to the scientific talks, the forum included virtual poster sessions with an introductory video or audio from the author and a chat window to submit questions and get feedback.

Participants were also encouraged to create virtual "hubs" at home to facilitate interactions. The forum even offered a virtual version

of its traditional 1-day mini meeting for graduate students and postdocs.

By all accounts, the virtual forum escaped most of the glitches that can plague a typical webinar. "My hat is off to LSI," Horányi says. "I was expecting a hell of a lot more technical problems. But they pulled it off."

Even so, he and other participants say the virtual conference was a pale imitation of the real thing. At previous forums, Horányi says, "You see your friends, you ask about their kids, and then the discussion flows into the science." He confesses that he participated much less this year—"2 hours a day would be a generous estimate." In

addition to the physical challenge of sitting at one's computer for hours on end, participants say that their day jobs competed for their attention. Schmidt estimates that some 150 to 200 people "attended" the forum at any one time.

Even without distractions, the quality of the interaction was much lower than in person. "I received a handful of short comments [from my talk] and had maybe one e-mail exchange," Horányi recalls. One scientist who didn't present this year—and who listened to only one talk after the fact—said that he much prefers an in-person meeting because

"you get a much better sense of how the audience is reacting to what you're saying, especially any negative feedback."

Schmidt agrees that a virtual meeting has serious limitations. "It funnels people into a very narrow setup," he admits. At the same time, he says that the institute welcomed the chance to test the idea because it relies on virtual interactions among institute members.

But there's a big difference between a virtual institute and a virtual meeting, says David Morrison, a senior scientist at the lunar institute and a former director of NASA's virtual Astrobiology Institute, also based at Ames. "I do not think the virtual approach works well for science conferences," says Morrison, who believes that a virtual institute makes sense only if collaborators also have regular face-to-face meetings throughout the year.

NASA hasn't decided on the format for next year's forum, Schmidt says, and its decision will be influenced by the responses to a survey asking participants what they liked and disliked. Despite the grumbling, Schmidt says one thing is already clear: "If virtual is the only option, they say they would rather have that than nothing." —JDM

Last year it was a performance. This year it meant **staring at myself ... thinking, 'Boy, am I getting old.'**

—MIHÁLY HORÁNYI,
UNIVERSITY OF COLORADO, BOULDER

The second blow is a 5% cut this year in the overall budgets of most agencies. That reduction, known as sequestration, kicked in this spring after the breakdown of a 2011 agreement between the White House and Congress to reduce the federal deficit.

Society officials say they have felt the effect of sequestration and tighter travel budgets. For example, some 14% fewer federal scientists attended last fall's AGU meeting, says the organization's CEO, Chris McEntee. In response, societies are trying to make meetings more enticing to both participants and those unable to attend in person. The revised fare includes Web-based features

such as electronic posters, live-streaming of some events, and archiving much of the content for later viewing.

But devotees say those new wrinkles are no substitute for what they consider the real thing: the chance to hear firsthand about new research, present their own findings, meet potential collaborators or mentors in person, and feel part of the tribe. Neural scientist Thomas Carew, dean of arts and sciences at New York University in New York City and a former president of the Society for Neuroscience (SfN), compares the experience of attending the society's annual meeting to a sporting event.



"You can feel the floor vibrate in the exhibit hall," he says about a meeting that last year attracted 28,574 people, good for 10th place on a ranking of the largest U.S. medical meetings. "There's a buzz that infuses the entire conference. For young scientists, it can be a transformative event in their careers."

Given all that a meeting offers, none of the society leaders anticipates switching to a virtual-only format in the foreseeable future, as NASA did this year with its annual Lunar Science Forum (see p. 75). "At least for me, there's nothing that could replace sitting and listening to a young scientist or a very prominent scientist explain his or her research to a group of people, all of whom are trained to ask hard questions and be skeptical," says Joseph McInerney, executive vice president of the American Society of Human Genetics, whose annual meeting draws about 7000 scientists.

For most societies, the annual meeting is also a moneymaker. Registration and exhibitor fees can contribute significantly to an organization's bottom line. SfN's annual meeting, for example, generated 43% of its overall revenue of \$29 million last year and netted \$3.8 million after expenses, according to the society's 2012 report.

The two major meetings put on by the Materials Research Society (MRS) each year do even better for the organization. Fueled by a record combined attendance of 13,750, the meetings produced 68% of the society's \$11 million in revenues last year, contributing \$4.6 million to its bottom line.

AGU's fall and spring meetings added \$1.5 million to the organization's coffers in 2011, a big help in a year in which overall expenses of \$39 million exceeded revenues by almost \$5 million.

Meetings That Flatter, but May Not Deliver

The e-mails come from Amber, Rainy, Dora, and Arlene. "How are you doing now?" some begin. "Hope this e-mail finds all the best on you." Flattering and solicitous and written in bewitchingly mangled English, the e-mails have the hallmarks of spam offering carnal pleasure—except they are actually far tamer. They are invitations to attend scientific meetings in China organized by a company that bills itself as the "World Leading Provider of Intelligence Exchanges in Life Sciences."

BIT Life Sciences, based in Dalian, a seaside city in Northeast China, stages conferences on a staggering array of topics, from vaccines and biodiversity to diabetes, cancer, cloud computing, HIV/AIDS, and algae. The meetings, which are often billed as an "Annual World Congress," sometimes coin names for new disciplines, such as "Endobolism" and "Drug Designology." BITeomics, the parent company, says it has 400 employees and holds at least 70 conferences a year that "tens of thousands of people" have attended since 2001.

Welcome to the bizarre world of what some call "predatory" conferences: scientific confabs, sometimes sparsely attended, that seem to come into being primarily to make money. Jeffrey Beall, a librarian at the University of Colorado, Denver, who monitors a subset of open-access journals that he calls "predatory," sees a similar phenomenon in BIT conferences. "They have the same conflict of interest as predatory publishers," he asserts. While predatory journals charge fees to publish papers, these conferences make money through registration fees that are bundled with charges for accommodation, meals, and program materials. (Typical bills run in the \$2000 range. BIT, which

stands for Bio Integration Technology, also has a subsidiary that offers to help book air flights, hotels, and tours.) "The more papers they accept, the more money they make," Beall says, as people with accepted talks are more likely to attend. While most scientific conferences have a similar financial equation, the vast majority are organized by nonprofits with members drawn from the scientific community, rigorously peer review submissions, and strictly limit the number of presentations. "Predatory" conferences, on the other hand, Beall says, "are accepting papers that

This stuff reminds me
of the *Who's Who*
business model.

—DEREK LOWE,
VERTEX PHARMACEUTICALS

may not be valid science: They bear the imprimatur of science even though they never go through the same quality control."

While BIT Congress claims to be "the largest-scale conference company in Asia Pacific," it has competition in what Beall says is an expanding industry. "They're just one in the landscape," he says. He has also taken aim at the OMICS Group, a company based in India that stages conferences and publishes open-access journals that Beall considers "predatory" (see p. 60). (OMICS strongly objects to being deemed "predatory" by Beall and has threatened to sue him for \$1 billion.)

In an e-mail to *Science*, Francis Wang, who works in the business development office of BIT Life Sciences, rejected the charge that the company stages predatory meetings and lowers the quality of scientific discourse. Their business, she stated, is information sharing: "We are a bridge to the professional world." Wang explained that the firm does not use spam or robots to send out e-mail invitations, and noted that only about 40% of participants use its travel subsidiary's services. She suggested that some of the criticism occurs because BIT Life Sciences reaches out to up-and-coming researchers. "We will try very hard to create more platforms to give young experts or junior scientists more visibility and encourage their motivation to engage in the competition in professional world," Wang stated.

Derek Lowe, a medicinal chemist at Vertex Pharmaceuticals in Cambridge, Massachusetts, has ridiculed BIT Life Sciences invitations on his blog, noting that he believes he's been invited to speak at meetings because he can breathe, speak, fill a slot on a schedule, and presumably pay the registration fee. "This stuff reminds me of the *Who's Who* business model," Lowe says. "You can be in this book of luminaries if you'll just pay for the book."

A typical e-mail from BIT begins by offering a slot to give an oral presentation or chair a session at a meeting that may not even intersect with your expertise. It will add that the program coordinator has invited you for your "invaluable experience and knowledge" or maybe because "you are an outstanding expert and have enjoyed great fame." The note will list other "world-class experts" and renowned speakers who have attended BIT conferences, including Nobel lau-

The allure of such profits, meanwhile, has created a growing number of “predatory” scientific meetings that appear to exist solely for making money (see p. 76).

Not all meetings are money spinners, of course. The general science meeting organized each year by the AAAS (which publishes *Science*) is not “anyone’s principal scientific meeting,” CEO Alan Leshner acknowledges. That secondary status limits how much the organization can charge registrants and exhibitors. As a result, he says, revenues are insufficient to cover many no-charge activities “that are central to our mission,”

Geophysical attraction. The American Geophysical Union’s fall meeting in San Francisco keeps growing.



reates. Program committees may well feature names you recognize from respected institutions. If you do not promptly accept, a reminder e-mail—“to ensure that you do not miss out”—is sure to follow. “Maybe there some problems with my mailbox and I haven’t received your kindly reply,” it will humbly suggest.

BIT conferences indeed have attracted Nobel laureates and other prominent speakers, some of whom vouch for the meetings they attended. “I did not learn much new, but the organization, et cetera, was OK,” says immunologist Rolf Zinkernagel, a Nobel-ist at the University of Zurich in Switzerland who gave a keynote lecture at an HIV/AIDS meeting in Tianjin in 2006. Alan Stone, a biochemist in London who previously chaired the International Working Group on Microbicides, attended the same conference and gave it a ringing endorsement. “The scientific presentations were well-chosen and the sessions I attended were effectively chaired,” he wrote the organizers in a review of the “excellent” meeting that he shared with *Science*. Malaria specialist David Warhurst, a professor emeritus at the London School of Hygiene & Tropical Medicine, gave a keynote speech at the 2nd Annual World Congress of Microbes-2012 and says it was a “valuable” meeting. “I had not been to an international meeting held under purely Chinese auspices there and enjoyed the experience,” Warhurst says. “I was able to meet some of the Chinese workers active in the fields I was interested in.”

**We are a
bridge to the
professional
world.**

—FRANCIS WANG,
BIT LIFE SCIENCES

Others, however, express serious misgivings about BIT. Some scientists—including officials such as Janet Woodcock, who directs the Center for Drug Evaluation and Research at the U.S. Food and Drug Administration, and Roger Glass, director of the Fogarty International Center at the U.S. National Institutes of Health—say they had no clue they were listed as advisory board members of a program committee until they were notified by *Science*. Immunologist Jeffrey Bluestone at the University of California, San Francisco, was billed as a “renewed” speaker for a meeting in 2011 that he did not agree to attend. “I have never and will never go to a BIT conference,” Bluestone says. “I have been trying for years to get them to stop including me on their lists.”

Attendees of some BIT conferences say they felt duped. “None of the colleagues that were supposed to be there were at the meeting,” says Mario Clerici, an immunologist from the University of Milan in Italy who chaired a session at a World AIDS Day meeting in 2011. “Ninety percent of the audience and of the speakers were Chinese, the rest a curious collection of people from exotic places. The general feeling was that of being stranded on a raft in the sea with a bunch of people who had never been sailing. In short: great opportunity to visit China, forget about science.”

Obstetrician/gynecologist Danny Schust of the University of Missouri, Columbia, says that he was honored by an invitation to chair a session

at a BIT conference and also curious to visit China. When he arrived at his session, there were only three other people there—including one from his own institution. “I don’t tell that story to many people because it’s kind of embarrassing,” Schust says. “I think lots of people are getting sucked into it. It kind of cheapens the whole research agenda.” To his surprise, BIT Life Sciences now lists him as a program committee advisory member of an upcoming meeting.

Wang told *Science* that BIT Life Sciences’ conferences list people as advisory board members only if they have agreed to serve that role. Speakers sometimes back out, she stated, which may explain why they are wrongly listed on a program. She acknowledged that on occasion, researchers receive invitations to speak at conferences outside their fields. “Some mismatched invitations can’t be avoided,” she wrote. Such issues are “the problems of a young organizer’s fast growth.” And she argued that it’s “absurd” that people would attend BIT Life Sciences meetings purely out of vanity. “Do you really believe, each year, those 10,000 professional professionals from more than 70 countries are all stupid? They are so easily hoaxed? And will they pay a good price and fly all the way to China just because they are flattered?”

At the end of some BIT Life Sciences invitations, researchers can opt out of future solicitations. “We will definitely unsubscribe requests from the bothered experts in our database,” Wang stated. The company is young, growing quickly, and trying to improve, she stressed: “In the garden of conferences, BIT is only a new flower bud with unyielding life power.”

—JON COHEN



Great Presenters

Lighting Up the Auditorium

Bonnie Bassler studies bacteria that live inside the gorgeous Hawaiian bobtail squid. The bacteria, by communicating with one another en masse, decide the proper time to light up like fireflies. The benefits are mutual: The bioluminescence helps camouflage the squid by eliminating its shadow on the ocean floor when moonlight bathes it from above, and the bacteria get nutrients from their host. It's a cool story, says Bassler, a molecular biologist

compares to individuals casting a vote and then making a group decision. She and her co-workers have shown that quorum sensing exists in all bacteria and controls myriad activities, from luminescence to toxin release.

The secret lives of bacteria makes for a compelling presentation, and Bassler does the topic justice. She says bacteria speak a lingua chemica with their own species, while also using a second Esperanto-like vocabulary that all bacteria use. If scientists figure out a way to muffle this chatter and in doing so hamper toxin release, she says, that could lead to new antibiotics. More profound still, quorum sensing informs us about human social interactions, like emotions rippling through a crowd. "How do you think we got those behaviors?" she asks, with mock incredulity that everyone doesn't know this. "It's because the bacteria invented them!"

Bassler, who looks like the late actress Gilda Radner with a splash of Lily Tomlin, loves an audience. "My job is to teach someone something they never knew, but it should not be like you're in a prisoner-of-war camp," she says. "I'm supposed to be teaching you but also entertaining you. You're giving me an hour of your time. It should be lively. We're on a hunt, it's a mystery, and it's amazing."

at Princeton University: "My bacteria glow in the dark—no human being doesn't like that."

Studying this symbiosis, Bassler's lab has deciphered quorum sensing, a system of chemical communication between bacteria that she com-



Bassler's Rules of Presentation

Stick to the big picture.

"We know this stuff in excruciating detail," she says. "You want to drive a metal stake through your head listening to our lab meetings."

On slides, use few words and make one point.

"People can read faster than I can talk," she says. "If I put the words there, I'm irrelevant."

Tell stories.

"These are detective stories with mini mysteries that all point to the same thing."

Don't strive to be the smartest person in the room.

"Sometimes people are like, 'Wow you don't sound scientific,'" she says. "The data are on the slide."

But the most important advice that Bassler has to offer has nothing at all to do with style: Prepare, prepare, prepare. "I've spent a gazillion hours to cull these nuggets from the morass," she says. **—J. C.**

such as a family science day and programs relating to international events and human rights.

Even societies with profitable meetings are doing what they can to make their meetings more accessible. The path is not always smooth, as Bob Braughler, virtual engagement manager at MRS, can attest.

The society's first major initiative was live streaming a 5-day symposium on energy and sustainability held during its November 2012 meeting in Boston. However, that decision ran afoul of scientists who balked at having their slides and words captured for posterity and made available to anyone. "We needed to go to each one of the presenters and request their permission," Braughler says, "but not everybody was willing to do that." The result was unsightly: a video with a 15-minute blank every time an author demurred.

The society's experience highlights the tension between wanting to open up a meeting to all while preserving the intellectual property rights attached to the content. Presenters were concerned about sharing information that might wind up in a journal article or become part of a patent application. "If my talk is going to be archived, then I can't transfer the copyright or file a patent," explains Husam Alshareef, a professor of materials science and engineering at King Abdullah University of Science and Technology in Jeddah, Saudi Arabia. "And MRS is petrified of being sued," says Alshareef, who is co-chair of the program committee for the society's 2014 fall meeting.

Until the society can work out those IP issues, it is proceeding with caution. For example, MRS has shifted its emphasis to what Braughler calls "video capture"—recording a session and then making the video available on demand, for free, to both attendees

Gut Instinct

When Larry Smarr pulls out a plastic model of his colon that he made with a 3D printer and simultaneously projects on a screen behind him a magnetic resonance image of his guts, it becomes abundantly clear that he believes a presentation benefits from a most personal touch.

Smarr started out as an astrophysicist and has followed an intriguing career arc from probing black holes to his own bowels. Three decades ago, he helped establish a network of supercomputer centers in the United States and was a cyber pioneer. (His grad student made the first Internet browser.) Today, the University of California, San Diego, professor runs the California Institute for Telecommunications and Information Technology (Calit2), a multidisciplinary center meshing nanotechnology with wireless communications, genomics, and computer science. Calit2, Smarr hopes, will become a force in personalized medicine—and his colon has become the centerpiece of a campaign to show the world how patients can take a more active role in their own health care by exploiting technological advances to collect genomic, biochemical, and physical data.

For several years, Smarr has intensely monitored his health, a preoccupation that in 2010 helped him diagnose, ahead of his doctors, inflammatory bowel disease. In a talk he has given everywhere from Harvard Medical School to the U.S. National Institutes of Health, Smarr shows startling graphics that chart myriad biochemical and physical perturbations in his body linked to what he believes is a condi-

tion with features resembling Crohn's disease and ulcerative colitis. He paid a company to measure blood markers that standard tests ignore, and, with the help of the J. Craig Venter Institute's genomic analysis of his fecal samples, he has documented how his body has killed off many beneficial bacterial species in his gut while allowing harmful ones to thrive. His plastic colon and the MRI scans fill out the sad picture of a gut gone haywire.

By blending systems biology and personal drama, Smarr's talks bowl audiences over. He speaks plainly; is passionate about his data (without bathing in self-pity about his disease); and makes the abstract concrete with his plastic colon, a prop that he passes around the audience. "I've given hundreds if not thousands of talks on so many different topics, and I've never had the kind of reaction I've had in the last few years," Smarr says. "When you talk about what's going on inside the human body, everyone relates."

A critical moment in his talk comes when he emphasizes that 90% of the DNA in our bodies is bacterial, and we can now sequence that foreign material to understand our health. "A lot of the reaction is, 'How did I miss the memo that 90% of



the cells in my body aren't human?' " Smarr says. "It's a moment of massive discovery essential to every single human on Earth. These moments don't come along more than a couple times in a century."

Few scientists have the luxury of drawing on data from their own bodies to captivate an audience, but one technique of Smarr's is widely applicable: Don't miss the forest for the trees. He suggests scientists imagine themselves as a baker—not a flour specialist—explaining how to make a fancy cake. "It's the integration of several ingredients over time," he says. "We aren't trained to think that way. We're trained just the opposite."

—J. C.

and those who agree to register. That platform gives the society more control over content before it is posted. "We'll probably live stream at least one event this fall," he says, while some two dozen symposia will be captured and put into the archives.

Likewise, the American Society for Microbiology drastically curtailed live streaming of last month's annual Interscience Conference on Antimicrobial Agents and Chemotherapy. The decision was based on a membership poll showing that 90% of the people who wanted online access to information from a meeting they could not attend chose the "archived with no live" option. "Live streaming is also the most costly option," says Connie Herndon, the society's director of meetings, speaking before the meeting, "so if our attendees don't really want it, then we'll probably reduce it to a minimal amount."

Logistics are another reason the venerable annual meeting is likely to persist. Organizers book meeting venues up to a decade

in advance, so any changes would necessarily take a long time to show up. "We're so big that we only fit into a few cities," says Nancy Todd, conference manager for the American Chemical Society (ACS), which holds large meetings in both the spring and the fall. Combining the two meetings, she says, would only worsen the space crunch.

But perhaps the biggest deterrent to change is the inherent conservatism of the community. "We've had two meetings [a year] since the beginning of time," Todd says. "It's what our members want." Neither federal cuts nor the Internet seem likely to change that winning formula for ACS and its sister organizations anytime soon.

—JEFFREY MERVIS

With reporting by Nisha Giridharan and Senah Yeboah-Sampong, 2013 Pitts Family Minority Science Writers interns.



Scholarly Communication: Cultural Contexts, Evolving Models

Diane Harley

Despite predictions that emerging technologies will transform how research is conducted, disseminated, and rewarded, why do we see so little actual shift in how scholars in the most competitive and aspirant institutions actually disseminate their research? I describe research on faculty values and needs in scholarly communication that confirm a number of conservative tendencies in publishing. These tendencies, influenced by tenure and promotion requirements, as well as disciplinary cultures, have both positive and negative consequences. Rigorous research could inform development of good practices and policies in academic publishing, as well as counter rhetoric concerning the future of peer review and scholarly communication.

Over two decades many have predicted that models of scholarly communication enabled by emerging technologies will transform how research is conducted, disseminated, and rewarded. The transformation would be driven by new generations of scholars weaned on file sharing, digital piracy, Facebook, Twitter, and yet-unrealized social media technologies. Prognostications about the future, however, are often devoid of empirical evidence useful in policy development. How can we achieve realistic publishing economies that serve the needs of scholars, as well as maintain quality in research output and institutional culture? In addition to laudable declarations and manifestos (1, 2), a critical mass of relevant and rigorous research would be welcome. This should include investigations into effective tenure and promotion (T&P) review practices in higher education globally; the role of bibliometrics and other mechanisms, such as open peer-review experiments, in providing a complement to traditional peer review; and ways to create sustainable and accessible publishing models that filter scholarship effectively, reliably, and in a way that cannot be gamed or abused.

Expectations for change in academic publishing have been influenced by pundits in the Internet industry, the open-source movement in computer programming, the rise of Web 2.0 social media tools, and the Wikipedia model of crowd-sourced production [e.g. (3)]. The ArXiv preprint server—used heavily by computationally based, high-paradigm, and low-commercial value fields—was often held up as a one-size-fits-all model that all disciplines could aspire to. “Big data” (4) and cyberinfrastructure (5) have promised unlimited data sharing and reuse, replicability and reproducibility in highly collaborative and distributed research environments. In this context, the journal article or monograph is con-

sidered to be a format un conducive to effective transfer of knowledge.

It has been suggested by some that making sense of the information overload resulting from this explosion of data and new publication forms could be ameliorated by new machine-generated algorithms (6), broadly referred to as alt-metrics (7). Adding to this mix is a proliferation of publication forms that take advantage of new media tools and shift the burden of payment for the production of scholarly literature from library subscriptions to authors via the author processing charge [gold open access (OA), e.g., Public Library of Science (PLOS) journals]. Most recently, we have seen a proliferation of gold OA megajournals following a variety of “publish then filter” models (e.g., *PLOS One*, *F1000Research*, and *SAGE Open*) that advertise fast publishing, article-level metrics, and open commenting as an alternative to stringent prepublication peer review by experts in a disciplinary community.

Reality Versus Rhetoric

Given this environment, why do we see so little actual shift in how scholars in the most competitive institutions, and aspirant institutions around the globe, actually disseminate their research at all stages? Our empirical research between 2005 and 2011 on cultural norms and disciplinary values in 12 disciplines (8, 9) revealed that individual imperatives for career self-interest, advancing the field, and receiving credit are often more powerful motivators in publishing decisions than the technological affordances of new media. There is an extraordinary reliance on peer-reviewed publications to aid T&P committees and external reviewers in the evaluation of scholarly work. Although we have heard many com-

plaints about peer review, publication imprimatur—with its associated peer review—was seen as an important proxy for assessing scholarly impact and importance. This reliance flows from the exponential growth, and resulting compartmentalization, of knowledge across the Academy and has the unwelcome result that individuals within a faculty can often no longer practically review the work of their peers or choose not to.

When compared with more ephemeral and lightly peer-reviewed “in-progress” research communications, archival peer-reviewed publications in established outlets have the heaviest weight in institutional evaluation, carry the highest prestige when making decisions about where to publish one’s own best research results, and are relied on as valuable quality filters for the proliferating mass of scholarly information available on the web.

Although the T&P process allows for disciplinary differences in type of scholarly product, a stellar record of high-impact publications continues to be the primary criterion for judging a successful scholar in the institutional peer-review process. Consequently, scholars choose outlets to publish their most important work based on three factors: (i) prestige (perceptions of rigor in peer review, selectivity, and “reputation”); (ii) relative speed to publication; and (iii) highest visibility within a target audience. When asked about more in-progress work—such as stand-alone cataloguing or curating, protocols, or ephemeral non-peer reviewed publications—informants said these would be credited, but could not substitute for nor be weighted as heavily as peer-reviewed “interpretive” work, as reflected in well-crafted arguments.

Despite the reliance on imprimatur as a proxy for peer review, most scholars claim that a candidate’s advancement dossier should be read closely and carefully by peers at the scholar’s home institution and that the advancement process at their institution can and should be supportive of (and not prejudiced by) nontraditional

“... individual imperatives for career self-interest, advancing the field, and receiving credit are often **more powerful motivators in publishing decisions** than the technological affordances of new media.”

publishing models, provided that peer review is strongly embedded and that there is evidence that the work is “ground-breaking” and “moves the field forward.”

Are committees seeing many examples that deviate from the established norms for a discipline, especially from young scholars? Not according to our informants, who represented all career stages. Evidence to suggest that “tech-savvy” young graduate students, postdoctoral scholars, and assistant professors are bypassing traditional publishing practices is not overwhelming. Young scholars

are products of intense socialization into an increasingly competitive academic working environment, reflected in the remarkably consistent advice given to pretenure scholars across fields: focus on publishing in the right venues and avoid spending too much time on competing activities. One would expect them to mimic the norms of their discipline, and they do so by following the lead of their mentors, even those established scholars who themselves exercise more freedom in publication choices but counsel conservative behavior in pretenure scholars.

The pace of archival publication in peer-reviewed outlets is growing not subsiding (10, 11). There is intense publication pressure on young scholars generally, as well as scholars at aspirant institutions globally. Imperatives to rise in league tables (academic rankings), in combination with research assessment-type exercises that tie government funding to research output, are significant drivers of this growth (12). The practices of competitive research universities have trickled down to aspirant institutions worldwide and translate into a growing glut of publications

Box 1. Future research.

Empirical research, especially social science research, can confirm good academic publishing practices and guide the future of scientific communication. We pose the questions below to structure that effort.

Determine primary indicators of effective T&P review practices across institutions and higher-education sectors internationally.

In order to identify successful models of T&P review, we need to understand which institutions rigorously engage in “best practices,” such as conducting “thick reviews” of a candidate’s dossier, limiting the number of publications that can be submitted in a dossier, or ignoring impact factors completely. Which rely too heavily on secondary indicators, such as impact factors?

One of our project’s more distinguished advisors from the biological sciences recommended the importance of senior scholars modeling and rewarding good practices in graduate students, postdocs, and pretenured faculty. How can we as a community of scholars institutionalize this advice?

The proliferation of international rankings presents challenges to researchers who are interested in how T&P practices vary across higher-education sectors and countries. These rankings schemes are being scrutinized for their effect on institutional missions (18). Do we know what are the actual costs (including social and opportunity costs) to teaching-intensive institutions of diverting academic labor from teaching to increasing research output, as measured primarily by publications and related impact factors?

How do research assessment exercises, league tables, and cash incentives to publish in high-impact journals affect the general quality and number of research publications?

Do top-down agendas from scholarly societies [e.g., (1)] or other non-university entities encourage the adoption of good practices? These organizations could track their members’ publishing patterns over time to determine relative effectiveness of the various factors influencing choices. Longitudinal and comparative ethnographies of carefully chosen universities could also provide a window into how, or if, resolutions, declarations, research assessment exercises, and institutional aspirations to advance in league tables are positive or negative levers for substantial change.

What are the effects on the academic enterprise of having some of the largest bibliometrics services controlled by publishers like Elsevier and Thomson Reuters (19)? Is the influence of these organizations on university rankings schemes hijacking a move to best practices by universities?

Assess whether bibliometrics or other mechanisms can evolve to filter scholarship effectively, reliably, and in a way that cannot be easily gamed or abused.

Scientific impact is a multidimensional concept that cannot be adequately measured by any one indicator (20, 21). What relation should the use of new metrics have to the more desirable qualitative “thick” reviews in academic promotion, grant competitions, and university rankings?



Are forms of hybrid quality-assessment models, which include traditional review and alt-metrics, more effective, and do they cost less, than the current system? Where is this being applied effectively? Are there models of successful crowd-sourced or alt-metric “peer review” applied to large data sets, and can they be applied outside of those cases as measures of quality (and be recognized in T&P decisions)?

Investigate ways to finance high-quality publication models while preserving the important work of most scholarly societies.

The majority of our informants made clear that scholarly societies are the natural communities of peers in a discipline and have played an important role in managing peer review and quality on multiple levels. Publication subscriptions are a key element of their operating budgets.

Will gold OA policies, such as those recommended in the UK Finch report (22), and the rise of gold OA megajournals be a positive development for the Academy, or do they represent vanity publishing that shifts costs onto authors? Will articles in these outlets be weighted as heavily in T&P decisions as the more traditional outlets and on what basis?

What new or existing financial publishing models can fund the activities of scholarly societies, and in what disciplines, while also increasing access to published scholarship? In response to calls for open access to federally funded research, a number of societies are implementing gold OA journals [e.g., (23)]. It would be useful to survey authors in various higher-education sectors and disciplines on how their promotions were affected by their publication choices in such outlets.

Opt-out OA resolutions at a variety of top-ranked institutions implicitly or explicitly recommend that all kinds of scholarship be considered in T&P decisions. Harvard and MIT, the first movers in this space, might systematically track shifts in publishing behavior of their faculty, especially among younger scholars.

Do we know if most readers want publications bursting with embedded data and linked commentary (with possibly exorbitant production costs), or smaller slices of curated scholarship, as represented in traditional publication formats? Surveys with good response rates and discipline-based ethnographic studies could help answer this and the other questions posed above.



and publication outlets, many of them low quality. This proliferation of outlets has placed a premium on distinguishing prestige outlets from those that are viewed as less stringently refereed, contradicting predictions that current models of publishing and of rating impact are going to be overturned in the foreseeable future.

How does this reality jibe with the hyperbole about Web 2.0 practices as transformative? We were particularly interested in questions of sharing research results. Although there was variation based on personality, our informants were clear that sharing early-stage research before it reaches a certain level of excellence is not widespread nor desirable. Working papers from scholars in top-tier institutions are unheard of in highly competitive fields, such as chemistry or molecular biology, both characterized by large grant funding, commercial potential of research, an extant quick turnaround time to publication, a surfeit of publications and outlets, and an overload of (or risks associated with relying upon) unvetted material. For astrophysicists and economists, outlets, such as arXiv and Social Science Research Network (SSRN), respectively, despite their utility as repositories for posting working papers and more ephemeral publications, do not replace the importance of formal archival publication because working papers are not recognized as, and were not necessarily intended to be, equivalent currency in T&P evaluations [e.g., (13)].

It is questionable whether new forms of scholarship outside of the traditional peer reviewed formats, including large data sets, will easily receive equivalent institutional credit. These outputs must be peer reviewed—and must be accompanied by an “interpretive” argument to receive such credit.

Who will conduct peer review of alternative forms of scholarship in an environment where publishing has become an “inflationary currency”? There is a near-continuous expenditure of activity on peer review by most scholars; the system is overloaded. As examples, two journals (14, 15) ceased publication of supplementary data because reviewers cannot spend the time necessary to review that material closely, and critical information on data or methods needed by readers can be lost in a giant, time-consuming “data dump.”

Will new forms of alt-metrics—that can “watch social media sites, newspapers, government policy documents and other sources for mentions of scholarly articles” (7)—provide reliable filters? It is too soon to tell how such tools might be valued in T&P and whether they can overcome the problem of being easily gamed. Could “open peer review,” where commentary is openly solicited and shared by random readers, colleagues, and sometimes editor-invited reviewers, be a panacea? We are not aware of any compelling evidence to suggest that a critical mass of scholars is offering comments in open journal forums, such as PLoS publications, prob-

ably for most of the same reasons identified in 2006 (16). Scholars usually read something once to determine its usefulness; given a choice, the version they will want to read will be the final, peer-reviewed one.

Research and Policy to Support Scholars

Although some might lament the conservative tendencies we describe, or offer only the promise of technical solutions to bring about cultural change, many of these tradition-driven behaviors serve important purposes in the life of scholarship. We should question whether jettisoning them wholesale could work against many of the values that most scholars agree are essential to good scholarship. Most scholars, however much they embrace the possibilities of new technologies in their research, are plagued with a lack of time; are in need of more, not fewer, filters and curated sources; and require a modicum of privacy and control over their in-progress research until it is ready for widespread public exposure. The drives to expose early ideas publicly, to rely on crowds for content creation and peer review, and to engage constantly with technology may not conduce the best scholarship.

How might we preserve the good and move away from some of the more negative parts of the current advancement system? Rather than demand unrealistic publication requirements of their members and give too much weight to impact factors and imprimatur, higher-education sectors globally should conduct thorough and context-appropriate internal institutional peer review of their members’ work, at the center of which should be a close reading and evaluation of a scholar’s overall contributions to a field in the context of each institution’s primary mission (1, 17). Even though some institutions have or may be considering policies that limit the number of publications that can be submitted in promotion cases, refuse to refer to a journal’s impact factor in assessing quality, or encourage members to publish less but more meaningfully, it is not clear, beyond the anecdotal, if these policies have any significant influence on actual behaviors.

Rigorous empirical research (see Box 1), especially from the social sciences, could inform the development of good practices in the academic publishing environment, as well as counter a surplus of rhetoric concerning the future of peer review and scholarly communication in digital environments.

References and Notes

- San Francisco Declaration on Research Assessment (2013); <http://am.ascb.org/dora/>.
- P. Bourne et al., Eds., “Force11Manifesto: Improving future research communication and e-scholarship” (Force 11, 2011); www.force11.org/white_paper.
- Y. Benkler, *The Wealth of Networks: How Social Production Transforms Markets and Freedom* (Yale Univ. Press, New Haven, CT, 2006).

- T. Hey, S. Tansley, K. Tolle, Eds., *The Fourth Paradigm: Data-Intensive Scientific Discovery* (Microsoft Research, Redmond, WA, 2004).
- D. Atkins et al., *Revolutionizing Science and Engineering Through Cyberinfrastructure: Report of the National Science Foundation Blue-Ribbon Advisory Panel on Cyberinfrastructure* (National Science Foundation, Washington, DC, 2003).
- M. Jensen, “The new metrics of scholarly authority,” *Chronicle Review* (The Chronicle of Higher Education online), 15 June 2007.
- Altmeter, www.altmeter.com.
- The core of research consisted of structured interviews with 160 faculty, administrators, librarians, and publishers across more than 45 “elite” research institutions largely in North America (and some in Western Europe), in over 12 disciplines. These interviews covered a variety of topics including tenure and promotion, sharing and publication, collaboration, data and resource use, and public engagement. Individuals were chosen through convenience sampling and a quota-informed system of snowball sampling to ensure that the informant pool represented a diversity of career stage and experience. The project resulted in multiple publications, offering comprehensive descriptions and analyses across much of the scholarly communication spectrum, including an in-depth investigation of peer review. These publications are available at The Future of Scholarly Communication. See <http://cshe.berkeley.edu/research/scholarlycommunication/index.htm>.
- D. Harley et al., *Assessing the Future Landscape of Scholarly Communication: An Exploration of Faculty Values and Needs in Seven Disciplines* [Center for Studies in Higher Education (CSHE), University of California, Berkeley, 2010].
- R. Bell, D. Hill, R. Lehming, *The Changing Research and Publication Environment in American Research Universities* (National Science Foundation, Washington, DC, 2007).
- M. Ware, M. Mabe, *The STM Report: An Overview of Scientific and Scholarly Journal Publishing* [International Association of Scientific, Technical, and Medical Publishers (STM), Oxford, 2009].
- C. Franzoni, G. Scellato, P. Stephan, *Science* **333**, 702–703 (2011).
- E. Henneken et al., *Learn. Publ.* **20**, 16–22 (2007).
- J. Maunsell, *J. Neurosci.* **30**, 10599–10600 (2010).
- C. Borowski, *J. Exp. Med.* **208**, 1337 (2011).
- S. Greaves et al., *Nature Web Debate: Peer Review* (2006); www.nature.com/nature/peerreview/debate/nature05535.html.
- D. Harley, S. K. Acord, *Peer Review in Academic Promotion and Publishing: Its Meaning, Locust, and Future* (Center for Studies in Higher Education, Univ. of California, Berkeley, 2011).
- A. Rauhvarges, *Global University Rankings and Their Impact* (European University Association, Brussels, 2011).
- K. Olds, *Globalhighered blog, Inside Higher Education* (2010); www.insidehighered.com/blogs/globalhighered/bibliometrics_global_rankings_and_transparency.
- J. Bollen, H. Van de Sompel, A. Hagberg, R. Chute, *PLoS ONE* **4**, e6022 (2009).
- R. Van Noorden, *Nature* **465**, 864–866 (2010).
- Report of the Working Group on Expanding Access to Published Research Findings (Finch Report) (Research Information Network, London, 2012); www.researchinfonet.org/wp-content/uploads/2012/06/Finch-Group-report-FINAL-VERSION.pdf.
- American Educational Research Association [press release], www.aera.net/Newsroom/News/AERAToLaunchOpenAccessJournal/tabid/14895/Default.aspx.

Acknowledgments: The work reported in this article was funded by the Andrew W. Mellon Foundation. D.H. thanks collaborators S. K. Acord, and C. J. King for comments on an earlier draft, as well as other team members, advisors, and anonymous informants.

23 July 2013; accepted 11 September 2013
10.1126/science.1243622



Integrative Annotation of Variants from 1092 Humans: Application to Cancer Genomics

Ekta Khurana *et al.*

Science **342**, (2013);

DOI: 10.1126/science.1235587

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/1235587.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.1235587.DC1.html>

This article **cites 90 articles**, 33 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/1235587.full.html#ref-list-1>

Integrative Annotation of Variants from 1092 Humans: Application to Cancer Genomics

Ekta Khurana, Yao Fu, Vincenza Colonna, Ximeng Jasmine Mu, Hyun Min Kang, Tuuli Lappalainen, Andrea Sboner, Lucas Lochovsky, Jieming Chen, Arif Harmanci, Jishnu Das, Alexej Abyzov, Suganthi Balasubramanian, Kathryn Beal, Dimple Chakravarty, Daniel Challis, Yuan Chen, Declan Clarke, Laura Clarke, Fiona Cunningham, Uday S. Evani, Paul Flicek, Robert Fragoza, Erik Garrison, Richard Gibbs, Zeynep H. Gümüş, Javier Herrero, Naoki Kitabayashi, Yong Kong, Kasper Lage, Vaja Liluashvili, Steven M. Lipkin, Daniel G. MacArthur, Gabor Marth, Donna Muzny, Tune H. Pers, Graham R. S. Ritchie, Jeffrey A. Rosenfeld, Cristina Sisu, Xiaomu Wei, Michael Wilson, Yali Xue, Fuli Yu, 1000 Genomes Project Consortium, Emmanouil T. Dermitzakis, Haiyuan Yu, Mark A. Rubin, Chris Tyler-Smith,* Mark Gerstein*

Introduction: Plummeting sequencing costs have led to a great increase in the number of personal genomes. Interpreting the large number of variants in them, particularly in noncoding regions, is a current challenge. This is especially the case for somatic variants in cancer genomes, a large proportion of which are noncoding.

Methods: We investigated patterns of selection in DNA elements from the ENCODE project using the full spectrum of variants from 1092 individuals in the 1000 Genomes Project (Phase 1), including single-nucleotide variants (SNVs), short insertions and deletions (indels), and structural variants (SVs). Although we analyzed broad functional annotations, such as all transcription-factor binding sites, we focused more on highly specific categories such as distal binding sites of factor *ZNF274*. The greater statistical power of the Phase 1 data set compared with earlier ones allowed us to differentiate the selective constraints on these categories. We also used connectivity information between elements from protein-protein-interaction and regulatory networks. We integrated all the information on selection to develop a workflow (FunSeq) to prioritize personal-genome variants on the basis of their deleterious impact. As a proof of principle, we experimentally validated and characterized a few candidate variants.

Results: We identified a specific subgroup of noncoding categories with almost as much selective constraint as coding genes: “ultra-sensitive” regions. We also uncovered a number of clear patterns of selection. Elements more consistently active across tissues and both maternal and paternal alleles (in terms of allele-specific activity) are under stronger selection. Variants disruptive because of mechanistic effects on transcription-factor binding (i.e. “motif-breakers”) are selected against. Higher network connectivity (i.e. for hubs) is associated with higher constraint. Additionally, many hub promoters and regulatory elements show evidence of recent positive selection. Overall, indels and SVs follow the same pattern as SNVs; however, there are notable exceptions. For instance, enhancers are enriched for SVs formed by nonallelic homologous recombination. We integrated these patterns of selection into the FunSeq prioritization workflow and applied it to cancer variants, because they present a strong contrast to inherited polymorphisms. In particular, application to ~90 cancer genomes (breast, prostate and medulloblastoma) reveals nearly a hundred candidate noncoding drivers.

Discussion: Our approach can be readily used to prioritize variants in cancer and is immediately applicable in a precision-medicine context. It can be further improved by incorporation of larger-scale population sequencing, better annotations, and expression data from large cohorts.

READ THE FULL ARTICLE ONLINE

<http://dx.doi.org/10.1126/science.1235587>



Cite this article as E. Khurana *et al.*, *Science* **342**, 1235587 (2013). DOI: 10.1126/science.1235587

FIGURES IN THE FULL ARTICLE

Fig. 1. Fraction of rare (DAF < 0.5%) SNPs.

Fig. 2. Fraction of rare SNPs in noncoding categories.

Fig. 3. SNPs in protein-protein interaction (PPI) network.

Fig. 4. Functional annotations of indels and SVs.

Fig. 5. Functional implications of positive selection.

Fig. 6. Functional interpretation of disease variants.

SUPPLEMENTARY MATERIALS

Materials and Methods

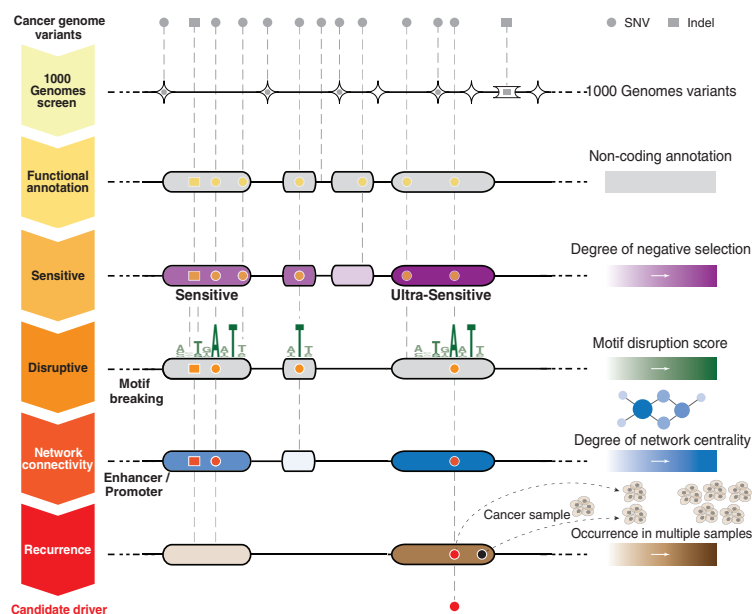
Supplementary Text

Fig. S1 to S29

Tables S1 to S12

References (49–90)

Data S1 to S7



Prioritization of candidate noncoding cancer drivers based on patterns of selection.

(Step 1) Filter somatic variants to exclude 1000 Genomes polymorphisms; (2) retain variants in noncoding annotations; (3) retain those in “sensitive” regions; (4) prioritize those disrupting a transcription-factor binding motif and (5) residing near the center of a biological network; (6) prioritize ones in annotation blocks mutated in multiple cancer samples.

The list of author affiliations is available in the full article online.

*Corresponding author. E-mail: cts@sanger.ac.uk (C.T.-S.); mark.gerstein@yale.edu (M.G.)

Integrative Annotation of Variants from 1092 Humans: Application to Cancer Genomics

Ekta Khurana,^{1,2*} Yao Fu,^{1*} Vincenza Colonna,^{3,4*} Xinmeng Jasmine Mu,^{1*} Hyun Min Kang,⁵ Tuuli Lappalainen,^{6,7,8} Andrea Sboner,^{9,10} Lucas Lochovsky,¹ Jieming Chen,^{1,11} Arif Harmanci,^{1,2} Jishnu Das,^{12,13} Alexej Abyzov,^{1,2} Suganthi Balasubramanian,^{1,2} Kathryn Beal,¹⁴ Dimple Chakravarty,⁹ Daniel Challis,¹⁵ Yuan Chen,³ Declan Clarke,¹⁶ Laura Clarke,¹⁴ Fiona Cunningham,¹⁴ Uday S. Evani,¹⁵ Paul Flicek,¹⁴ Robert Fragoza,^{13,17} Erik Garrison,¹⁸ Richard Gibbs,¹⁵ Zeynep H. Gümüş,^{10,19} Javier Herrero,¹⁴ Naoki Kitabayashi,⁹ Yong Kong,^{2,20} Kasper Lage,^{21,22,23,24,25} Vaja Liliashvili,^{10,19} Steven M. Lipkin,²⁶ Daniel G. MacArthur,^{22,27} Gabor Marth,¹⁸ Donna Muzny,¹⁵ Tune H. Pers,^{24,28,29} Graham R. S. Ritchie,¹⁴ Jeffrey A. Rosenfeld,^{30,31,32} Cristina Sisu,^{1,2} Xiaomu Wei,^{13,33} Michael Wilson,^{1,33} Yali Xue,³ Fuli Yu,¹⁵ 1000 Genomes Project Consortium,[†] Emmanouil T. Dermitzakis,^{6,7,8} Haiyuan Yu,^{12,13} Mark A. Rubin,⁹ Chris Tyler-Smith,^{3,‡} Mark Gerstein^{1,2,34,‡}

Interpreting variants, especially noncoding ones, in the increasing number of personal genomes is challenging. We used patterns of polymorphisms in functionally annotated regions in 1092 humans to identify deleterious variants; then we experimentally validated candidates. We analyzed both coding and noncoding regions, with the former corroborating the latter. We found regions particularly sensitive to mutations (“ultrasensitive”) and variants that are disruptive because of mechanistic effects on transcription-factor binding (that is, “motif-breakers”). We also found variants in regions with higher network centrality tend to be deleterious. Insertions and deletions followed a similar pattern to single-nucleotide variants, with some notable exceptions (e.g., certain deletions and enhancers). On the basis of these patterns, we developed a computational tool (FunSeq), whose application to ~90 cancer genomes reveals nearly a hundred candidate noncoding drivers.

Whole-genome sequencing has revealed millions of variants per individual. However, the functional implications of the vast majority of these variants remain poorly understood (1). It is well established that variants in protein-coding genes play a crucial role in human disease. Although it is known that noncoding regions are under negative selection and that variants in them have been linked to disease, their role is generally less well understood (2–9).

In particular, whereas some studies have demonstrated a link between common variants from genome-wide association studies (GWASs) and regulatory regions (2, 3), the deleterious effects of rare inherited variants and somatic cancer mutations in noncoding regions have not been explored in a genome-wide fashion. Recently, three studies reported noncoding driver mutations in the *TERT* promoter in multiple tumor types, including melanomas and gliomas (10–12). In light of these studies and the growing availability of whole-genome cancer sequencing (13–20), an integrated framework facilitating functional interpretation of noncoding variants would be useful.

One may think to identify noncoding regions under strong selection purely through mammalian sequence conservation, and ultraconserved elements have been found in this fashion (21). However, signatures of purifying selection identified by using population-variation data could provide better insights into the importance of a

genomic region in humans than evolutionary conservation. This is because many regions of the genome show human-specific purifying selection, whereas other regions conserved across mammals show a lack of functional activity and selection in humans (7). Thus, identifying the specific elements under particularly strong purifying selection among humans could provide novel insights.

Besides single-nucleotide polymorphisms (SNPs), the human genome also contains other variants, including small insertions and deletions (indels) and larger structural variants (SVs) (22). They account for more nucleotide differences among humans than SNPs; hence, an understanding of their relationship with functional elements is crucial (23).

We used the full range of sequence polymorphisms (ranging from SNPs to SVs) from 1092 humans to study patterns of selection in various functional categories, especially noncoding regulatory regions (24). We identified specific genomic regions where variants are more likely to have strong phenotypic impact. The list of these regions includes groups of coding genes and specific sites within them and, importantly, particular noncoding elements. By further comparing patterns of polymorphisms with somatic mutations, we show how this list can aid in the identification of cancer drivers. We used multiple experimental methods for validation, including yeast two-hybrid experiments, Sanger sequencing of independent

cancer samples, and relevant gene-expression measurements. Furthermore, we provide a software tool that allows researchers to prioritize noncoding variants in disease studies.

Genomic Elements Under Strong Purifying Selection: Ultrasensitive Regions

Enrichment of rare variants can be used to estimate the strength of purifying selection in different functional categories (24). As expected, we found that having variants from 1092 individuals allowed us to detect specific functional categories under strong purifying selection with greater

¹Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT 06520, USA. ²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA. ³Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK. ⁴Institute of Genetics and Biophysics, National Research Council (CNR), 80131 Naples, Italy. ⁵Center for Statistical Genetics, Biostatistics, University of Michigan, Ann Arbor, MI 48109, USA. ⁶Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva, Switzerland. ⁷Institute for Genetics and Genomics in Geneva (iGE3), University of Geneva, 1211 Geneva, Switzerland. ⁸Swiss Institute of Bioinformatics, 1211 Geneva, Switzerland. ⁹Institute for Precision Medicine and the Department of Pathology and Laboratory Medicine, Weill Cornell Medical College and New York–Presbyterian Hospital, New York, NY 10065, USA. ¹⁰The HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medical College, New York, NY 10021, USA. ¹¹Integrated Graduate Program in Physical and Engineering Biology, Yale University, New Haven, CT 06520, USA. ¹²Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, NY 14853, USA. ¹³Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA. ¹⁴European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK. ¹⁵Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX 77030, USA. ¹⁶Department of Chemistry, Yale University, New Haven, CT 06520, USA. ¹⁷Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA. ¹⁸Department of Biology, Boston College, Chestnut Hill, MA 02467, USA. ¹⁹Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY, 10065, USA. ²⁰Keck Biotechnology Resource Laboratory, Yale University, New Haven, CT 06511, USA. ²¹Pediatric Surgical Research Laboratories, Massachusetts General Hospital for Children, Massachusetts General Hospital, Boston, MA 02114, USA. ²²Analytical and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA 02114, USA. ²³Harvard Medical School, Boston, MA 02115, USA. ²⁴Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark. ²⁵Center for Protein Research, University of Copenhagen, Copenhagen, Denmark. ²⁶Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA. ²⁷Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology (MIT), Cambridge, MA 02142, USA. ²⁸Division of Endocrinology and Center for Basic and Translational Obesity Research, Children’s Hospital, Boston, MA 02115, USA. ²⁹Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. ³⁰Department of Medicine, Rutgers New Jersey Medical School, Newark, NJ 07101, USA. ³¹IST/High Performance and Research Computing, Rutgers University Newark, NJ 07101, USA. ³²Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, NY 10024, USA. ³³Child Study Center, Yale University, New Haven, CT 06520, USA. ³⁴Department of Computer Science, Yale University, New Haven, CT 06520, USA.

*These authors contributed equally to this work.

†A full list of participants and institutions is available in the supplementary materials.

‡Corresponding author. E-mail: cts@sanger.ac.uk (C.T.-S.); mark.gerstein@yale.edu (M.G.)

power than previously possible (2, 7, 9). In particular, the increased number of samples provided a better estimate of allele frequencies, making possible the measurement of differential selective constraints between specific categories [e.g., between motifs of transcription-factor (TF) families HMG and MADs box] (figs. S4 and S5).

Estimates of purifying selection obtained by using enrichment of rare nonsynonymous SNPs (derived allele frequency or DAF < 0.5%) showed that different gene categories exhibit differential selection consistent with their known phenotypic consequences (data S1). Genes tolerant of loss-of-function (LoF) mutations are under the weakest selection, whereas cancer-causal genes are under the strongest (Fig. 1A and table S1). GWAS genes associated with complex disorders lie in between these extremes, consistent with the presence of common genetic variants in them.

We then analyzed selective constraints in non-coding regions, trying to find elements under very strong selection (i.e., with a fraction of rare variants similar to that of coding genes, ~67%). We first estimated the strength of negative selection in broad categories [e.g., in all TF binding sites (TFBSs), deoxyribonuclease I (DNaseI)-hypersensitive sites (DHSs), noncoding RNAs (ncRNAs), and enhancers] (Fig. 2A). As observed previously, most of these categories show slight but statistically significant enrichment of rare SNPs compared with the genomic average; in contrast, pseudogenes demonstrate a depletion (Fig. 2A and data S2) (2).

We further divided the broad categories into 677 high-resolution ones. These span various genomic features likely to influence the extent of selection acting on the element. For example, TFBSs of different TF families are divided into proximal versus distal and cell-line-specific versus -nonspecific (fig. S7). We find heterogeneous degrees of negative selection for specific categories (Fig. 2B and data S2). For instance, core motifs in the binding sites of TF families HMG and Forkhead are under particularly strong selection, whereas those in the CBF-NFY family do not exhibit selective constraints (relative to

the genomic average) (Fig. 2B). Among all the pseudogenes, polymorphic ones have the highest fraction of rare alleles, consistent with their functional coding roles in some individuals (25). Overall, we found that 102 of the 677 categories show statistically significant selective constraints (data S2) (figs. S8 to S10).

Among these 102 categories, we defined the top ones covering ~0.02% and ~0.4% of the genome as ultrasensitive and sensitive, respectively (fig. S11) (data S3). Thus, these regions were defined such that they possess a high fraction of rare variants comparable to that for coding sequences (67.2% for coding and 65.7% for ultrasensitive) (Fig. 2C). We validated the rare variants in them by comparison with Complete Genomics data. Sensitive regions include binding sites of some chromatin and general TFs (e.g., *BRF1* and *FAM48A*) and core motifs of some important TF families (e.g., JUN, HMG, Forkhead, and GATA). For some TFs, there is a strong difference between proximal and distal binding sites—for example, for *ZNF274*, proximal binding sites are under strong selection and belong to the ultrasensitive category, whereas distal sites are not under negative selection.

In order to validate the functional importance of sensitive and ultrasensitive regions, we examined the presence of inherited disease-causing mutations from HGMD (Human Gene Mutation Database) in them (26). We found ~40- and ~400-fold enrichment of disease-causing mutations in sensitive and ultrasensitive regions, respectively (compared with the entire noncoding sequence, $P < 2.2 \times 10^{-16}$) (Fig. 2E). Thus, these documented disease-causing variants provide independent validation for the functional importance of sensitive regions. As a specific example, the disease congenital erythropoietic porphyria is caused by disruption of a binding site classified as sensitive (the *GATA1* motif upstream of uroporphyrinogen-III synthase) (27). Similarly, the well-known disease-causing ncRNA *RMRP* is in the binding site of *BRF2*, classified as ultrasensitive (28).

Purifying Selection and Other Aspects of Regulatory Regions

We analyzed sites at which SNPs break or conserve core-binding motifs. As expected, we found that disruptive motif-breaking SNPs are significantly enriched for rare alleles compared with motif-conserving ones ($P < 2.2 \times 10^{-16}$; Fig. 2D; a motif-breaking SNP is defined as a change that decreases the matching score in the motif position weight matrix). This result is over all TF families; moreover, we find the difference between constraints on motif-breaking versus -conserving SNPs varies considerably for different TF families, possibly reflecting differences in the topology of their DNA binding domains (data S4).

We also found that expression quantitative trait loci (eQTLs) are enriched in the binding sites of many TF families (Fig. 2B); the association of TF binding and gene expression at these loci provides a plausible explanation for their phenotypic effects.

An analysis of SNPs from a personal genome (NA12878) exhibiting allele-specific TF binding in chromatin immunoprecipitation sequencing (ChIP-Seq) data or allele-specific expression in RNA-seq data (with the allele-specific “activity” tagging a difference between maternal and paternal chromosomes at the genomic region in question) showed that these sites are depleted for rare variants (relative to a matched control) (Fig. 2F). This suggests that regions where differential allelic activity is not observed may be under stronger purifying selection (29).

In a similar fashion, we found that core-motif regions bound in a “ubiquitous manner” (i.e., where differential cell-type-specific binding is not observed) are under stronger selection than those bound by TFs in a single cell line (data S2), consistent with the greater functional importance of ubiquitously bound regions. In relation to this, we further examined how selective constraints vary among coding genes and DHSs with tissue-specific activity (Fig. 1B). We found there are pronounced differences between tissues: For example, genes with ovary- and brain-specific ex-

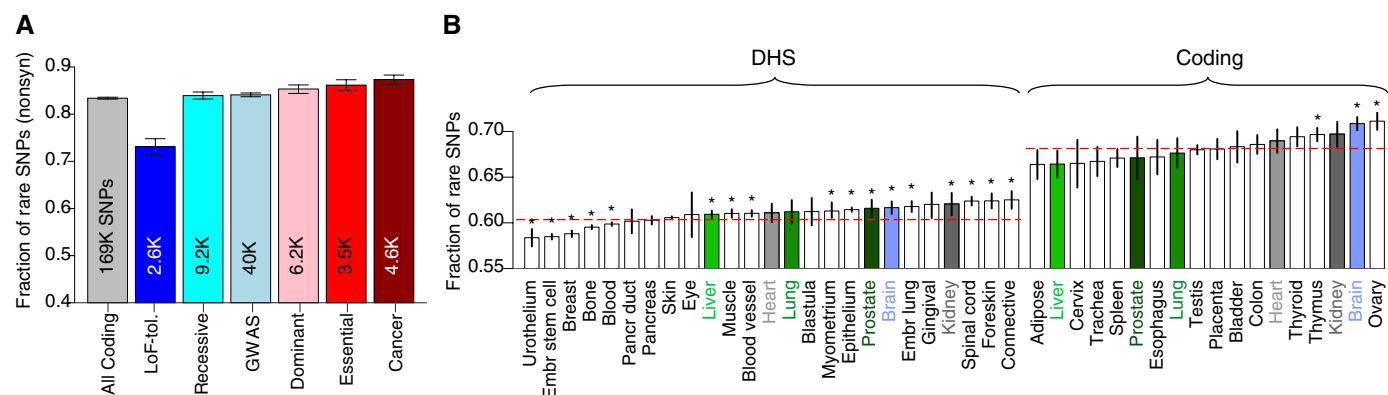


Fig. 1. Fraction of rare (DAF < 0.5%) SNPs. (A) In various gene categories. Total number of SNPs in each category shown. **(B)** In noncoding DHSs and coding genes, which show tissue-specific behavior. Matching tissues for which both DHS and gene expression data are available shown in same colors: shades of green

for endodermal, gray for mesodermal, and blue for ectodermal origin of tissues. Red dotted lines show the total fraction for all DHSs and coding genes. Asterisks show significant depletion or enrichment after multiple-hypothesis correction. Error bars in both (A) and (B) denote 95% binomial confidence intervals.

pression are under significantly stronger selection than the average across all tissues (Fig. 1B and table S4). Similarly, some DHSs are under significantly stronger selection, whereas others are under relaxed constraints relative to the average (brain- and kidney-specific versus urothelium- and breast-specific, respectively; Fig. 1B and table S4). Last, matched expression and DHS data for six tissues indicate that purifying selection in tissue-specific genes and their corresponding regulatory regions is likely correlated (fig S15). Thus, our results suggest that the deleteriousness of both coding and regulatory variants depends on the tissues they affect.

Purifying Selection in the Interactome and Regulome

We found a significant positive correlation between the fraction of rare SNPs and the degree centrality of genes in networks: physical protein-protein interaction (PPI) ($\rho = 0.15$; $P < 2.2 \times 10^{-16}$) and regulatory ($\rho = 0.07$; $P = 6.8 \times 10^{-08}$). Thus, consistent with previous studies, we found

that hub genes tend to be under stronger negative selection (29–31). Indeed, centralities of different gene categories in the PPI network follow the same trend as differential selective constraints on them: Cancer-causal genes show the highest connectivity, and LoF-tolerant genes, the least, with GWAS genes in the middle (Figs. 1A and 3A). These results indicate that the interactions of a gene likely influence the selection acting on it.

Hub proteins tend to have more interaction interfaces in the PPI network (31). A corollary of this is that interaction interfaces are themselves under strong selection, in turn leading to stronger constraints on hub proteins. Indeed, we found that SNPs disrupting interaction interfaces are enriched for rare alleles ($P < 2.2 \times 10^{-16}$) (Fig. 3B). To further corroborate this, we tested a specific case, the Wiskott-Aldrich syndrome protein (*WASP*), using yeast two-hybrid (Y2H) experiments (32). All of the three tested single-nucleotide variants (SNVs) at *WASP* interaction interfaces disrupted its interactions with other proteins (Fig. 3C). We observed similar behavior for

two other proteins: Mutations at their interfaces disrupted specific protein interactions (fig. S16).

Relationship of Functional Elements with Indels and Larger SVs

We analyzed the association of functional annotations with small indels [<50 base pairs (bp)] and large SVs (deletions). Similar to the results for nonsynonymous SNPs, we found that genes linked with diseases show stronger selection against indels whereas LoF-tolerant genes show weaker constraints (relative to all genes), with a consistent trend for indels overall and frame-shift indels, in particular (Fig. 4A, fig. S17, and table S1).

The wide range of SV sizes (~ 50 bp to ~ 1 Mb) leads to their diverse modes of intersection with functional elements; for example, a single SV breakpoint can split an element, a smaller SV can cut out a portion of a single element, and a large SV can engulf an entire element. To analyze the diverse effects of SVs, we computed the enrichment or depletion of SVs overlapping each

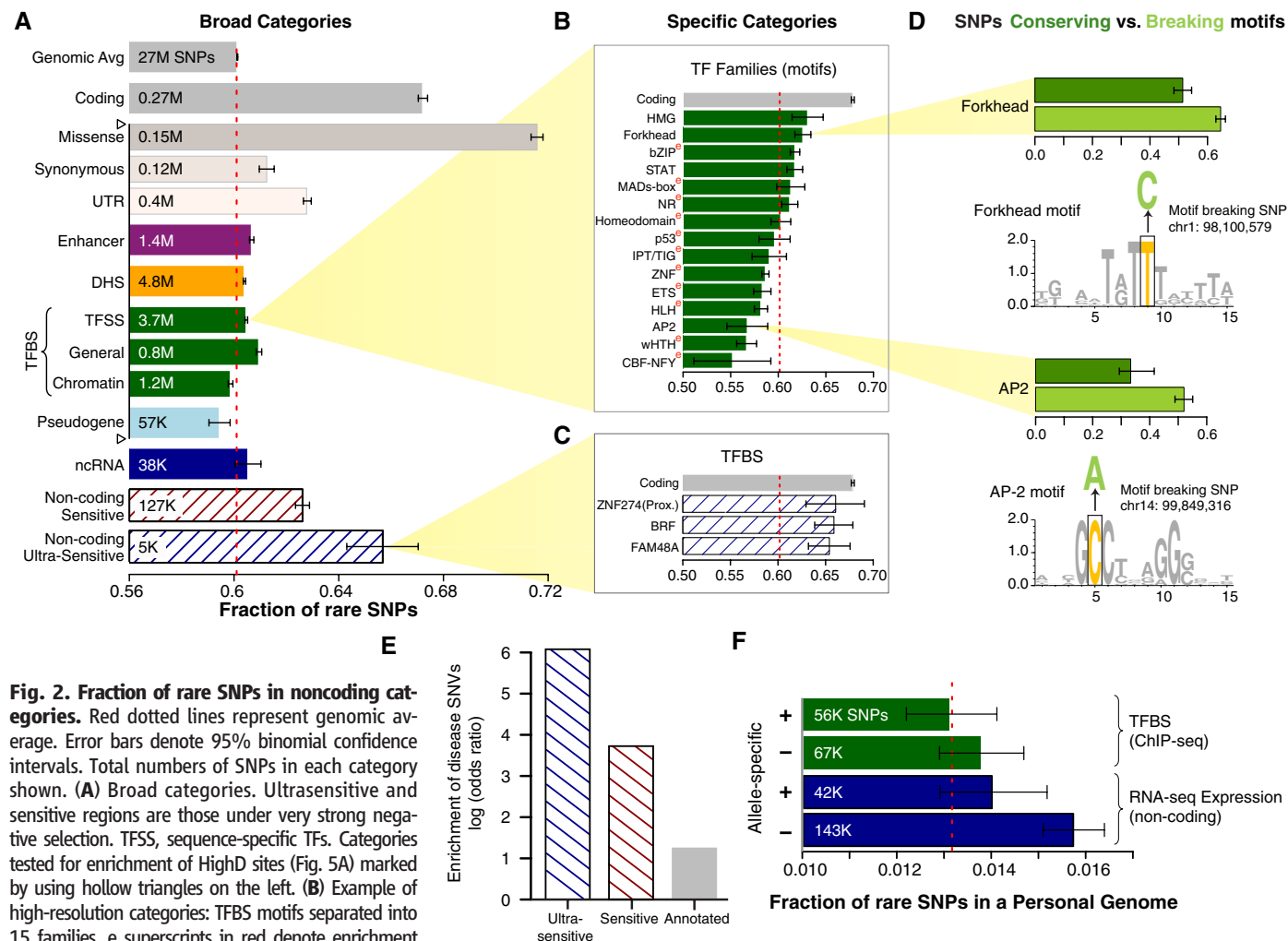


Fig. 2. Fraction of rare SNPs in noncoding categories. Red dotted lines represent genomic average. Error bars denote 95% binomial confidence intervals. Total numbers of SNPs in each category shown. (A) Broad categories. Ultrasensitive and sensitive regions are those under very strong negative selection. TFSS, sequence-specific TFs. Categories tested for enrichment of HighD sites (Fig. 5A) marked by using hollow triangles on the left. (B) Example of high-resolution categories: TFBS motifs separated into 15 families. e superscripts in red denote enrichment of eQTLs in TFBSs of specific families. (C) Examples of TFBSs included in ultrasensitive category. (D) SNPs breaking TF motifs show an excess of rare alleles compared with those conserving them. Representative motifs for two families are shown. (E) Enrichment of HGMD regulatory disease-causing

mutations in ultrasensitive, sensitive, and annotated regions compared with all noncoding regions. (F) SNPs not exhibiting allele-specific behavior (–) are enriched in rare alleles compared with SNPs exhibiting allele-specific behavior (+).

functional category relative to a randomized control. As expected, we found that genic regions [coding sequences, untranslated regions (UTRs), and introns] are depleted for SVs, suggesting SVs affecting gene function are deleterious (Fig. 4B) (22). However, when we broke down the mode of SV intersection with genes into partial versus whole (an SV breakpoint splitting a gene versus an SV engulfing a whole gene), we unexpectedly found that SVs are enriched for whole-but depleted for partial-gene overlap. This suggests that partial-gene overlap is under stronger selection than whole-gene overlap, possibly because whole-gene deletions may be compensated by duplications. Furthermore, another category of gene-related elements, pseudogenes, are enriched for SVs, consistent with their formation mechanism involving either duplication or retrotransposition.

In relation to nongenic elements, we found that SVs tend to be depleted in regulatory elements such as binding-site motifs and enhancers (Fig. 4B), consistent with our expectations from SNPs. However, enhancer elements are enriched for SVs formed by nonallelic homologous recombination (NAHR). This observation is further supported by the high signal of activating histone marks associated with enhancers (e.g., H3K4me1) around NAHR breakpoints (Fig. 4C and fig. S18). The association of enhancers and NAHR deletions may be explained by the three-dimensional structure of chromatin bringing enhancer elements into close proximity with the gene transcription start site (via DNA “looping”). If these two “non-allelic” loci contain homologous sequences, it would be favorable for NAHR to occur.

Functional Implications of Positive Selection Among Human Populations

Negative selection is widespread in the genome; nevertheless, some positions within negatively selected regions also experience positive selection (33–36). We have previously identified and validated one category of variants that are strong candidates for positive selection: sites where continental populations show extreme differences in DAF (HighD sites) (24). By analyzing these HighD sites, we are focusing on positive selection under the classic selective-sweep model (37). Positive selection via other modes (such as selection on standing variation) likely also played a major role in recent human evolution (38). Nonetheless, functional annotation of HighD sites can provide important insights about recent adaptations (39).

We examined positive selection in the same fashion as we have done for negative selection: in coding genes, noncoding regulatory elements, and networks of gene interactions. The functional analysis of positive selection using highly differentiated sites is limited to SNPs, because of the low numbers of such indels and SVs in functional elements.

We observed enrichment of HighD sites in UTRs and missense SNPs in coding regions (Fig. 5A). Next, we observed that some disease

gene groups (Online Mendelian Inheritance in Man, HGMD, and GWAS) are enriched for HighD SNPs (fig. S20). Mutations in disease genes are likely to have strong phenotypic impact; thus, it is possible that some of these mutations confer advantage for local adaptation. For example, whereas LoF mutations in *ABCA12* lead to the severe skin disorder harlequin ichthyosis (40), we found that a SNP within the second intron of this gene is a HighD site (DAF > 90% in Europe and East Asia; 13% in Africa), possibly reflecting adaptations of the skin to levels of sunlight outside of Africa.

Similar to our analysis of negative selection, we analyzed the enrichment of HighD sites in broad and specific noncoding categories, finding significant enrichment in many noncoding categories (Fig. 5A). These enriched categories include DHSs (particularly distal ones) and binding sites of sequence-specific TFs (specifically those

in ZNF and NR families). Out of the seven enriched categories, five are also under significant negative selection (Figs. 2A and 5A and data S2). Thus, even though an entire category might be under negative selection, some particular sites within it can be targets of positive selection. In this respect, our results are consistent with previous studies for missense SNPs: Overall they are under strong negative selection, but a small group of them have been targets of positive selection (36).

We found that, as expected, coding genes with HighD SNPs tend to have lower degree centrality in both PPI and regulatory networks (although the small number of these cases does not produce statistical significance) (Fig. 5B and fig. S21) (41). In an opposite trend to genes (where positive selection occurs on the network periphery), HighD sites in TFBSs tend to occur in hub promoters ($P = 0.02$ with 23 promoters and $P = 3.2 \times 10^{-03}$ with

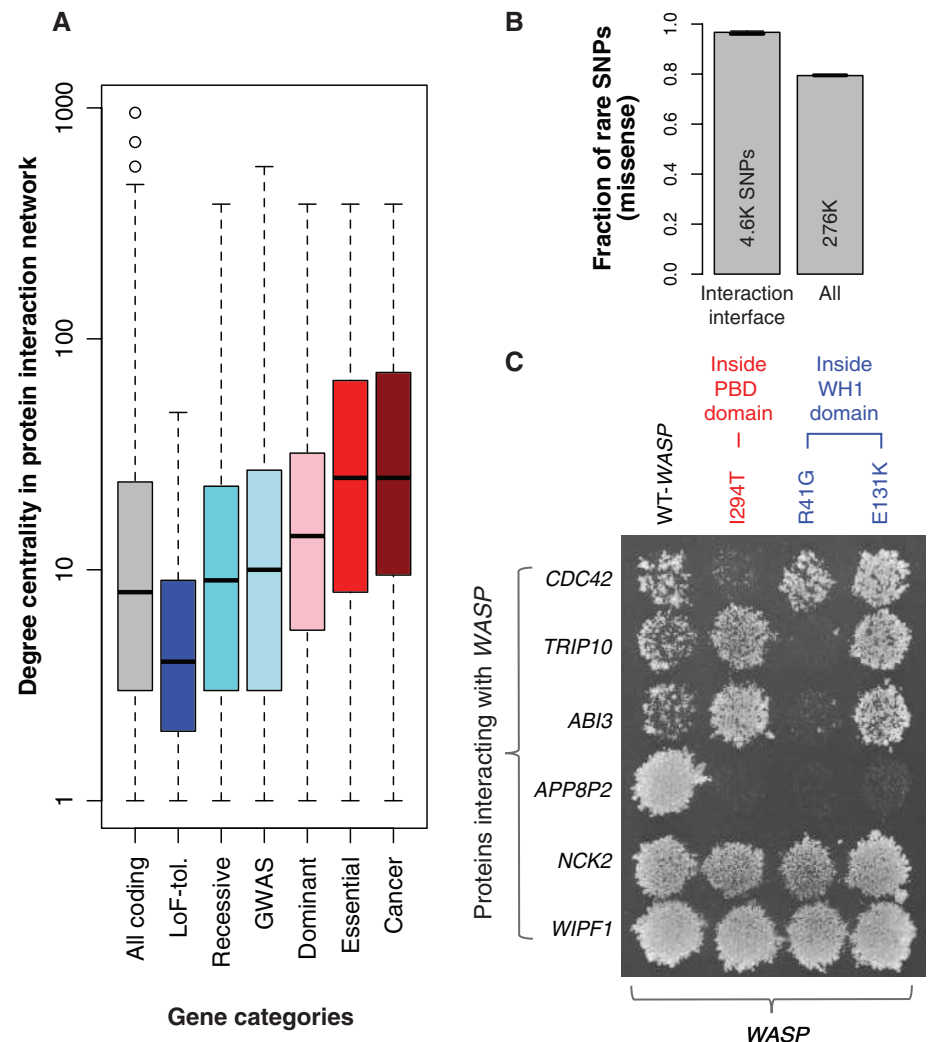


Fig. 3. SNPs in protein-protein interaction (PPI) network. (A) Degree centrality of coding-gene categories in PPI network. (B) Fraction of rare missense SNPs at protein-interaction interfaces is higher than all rare missense SNPs (error bars show 95% binomial confidence intervals; total number of SNPs also shown). (C) Effects of SNVs at interaction interfaces on interactions of WASP with other proteins tested by Y2H experiments. Wild-type (WT) WASP interacts with all proteins shown, whereas each missense SNV disrupts its interaction with at least one protein.

37 proximal TFBSs) (Fig. 5B). It was previously proposed that mutations in cis elements in regulatory networks may play an important role in development (42, 43); our study supports this by suggesting that some hub promoters may have undergone recent adaptive evolution.

Contrasting Patterns of Somatic Mutations with Inherited Variants

After analyzing inherited polymorphisms in functional elements, we examined somatic variants. Because somatic variants from diverse tumors exhibit different sets of properties, we analyzed variants from a wide range of cancer types: prostate, breast, and medulloblastoma (17, 19, 20). We found that

~99% of somatic SNVs occur in noncoding regions, including TFBSs, ncRNAs, and pseudogenes (fig. S22).

Analysis of matched tumor and normal tissues from the same individuals showed that somatic variants tend to be enriched for missense (~5×), LoF (~14×), sensitive (~1.2×), and ultrasensitive (~2×) variants (Fig. 6A, fig. S24, and table S6). Consistent with this trend, we found higher TF-motif-breaking/conserving ratios for somatic variants compared with germline ones across many different samples and cancer types (~3 for somatic versus ~1.4 for germline) (Fig. 6B and table S7). Thus, somatic-cancer variants are generally enriched for functionally deleterious mutations.

This enrichment of functionally deleterious mutations among somatic variants is understandable because they are not under organism-level natural selection (unlike inherited-disease mutations, including GWAS variants). Indeed, among all somatic mutations, those most deviating from patterns of natural polymorphisms are the most likely to be cancer drivers. Consistent with this, our analysis has shown that, among all disease mutations, those causing cancer occur in genes under strongest negative selection (and with highest network connectivity) (Figs. 1A and 3A). Thus, we argue that somatic variants in the noncoding elements under strongest selection are the most likely to be cancer drivers.

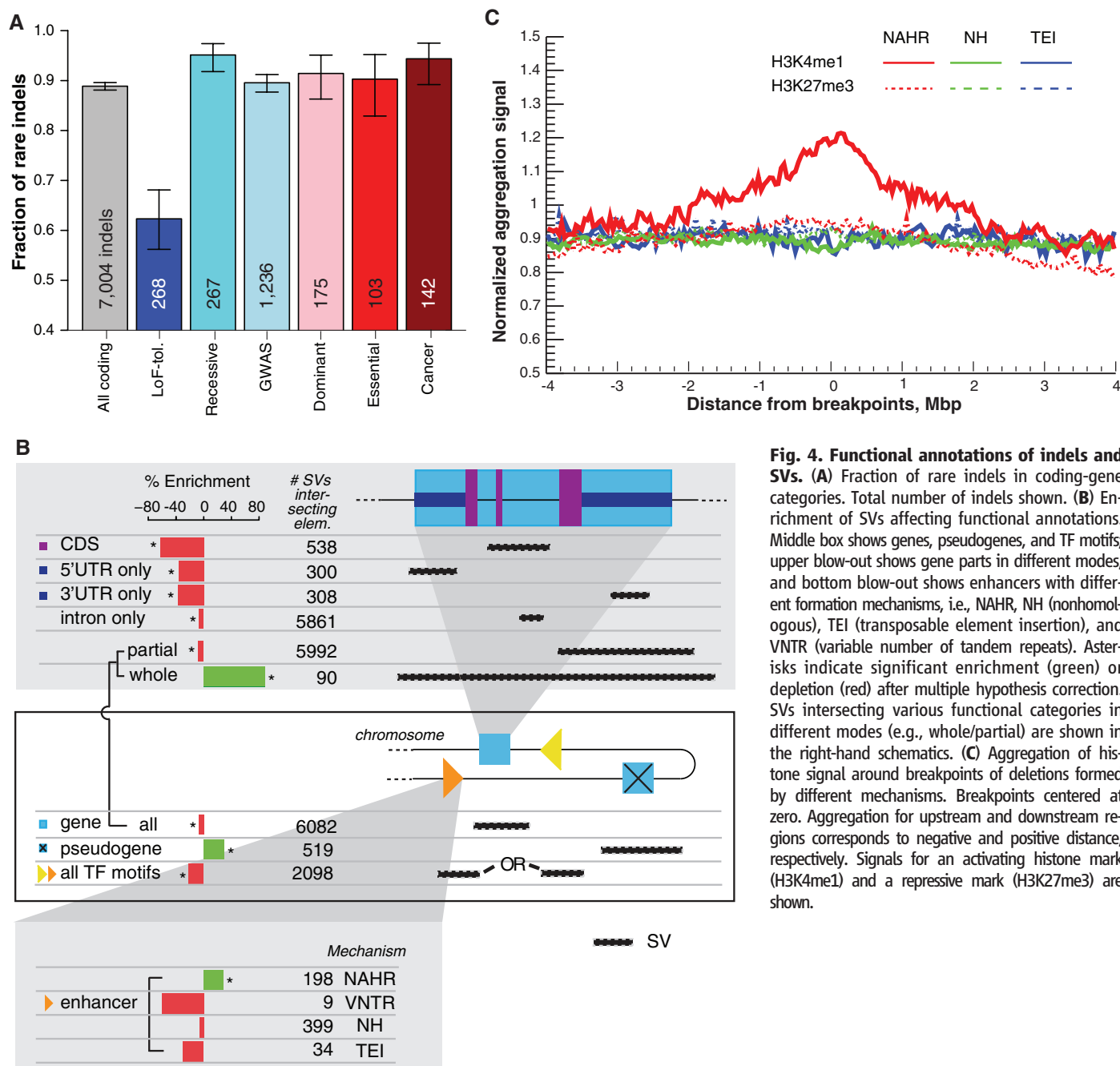


Fig. 4. Functional annotations of indels and SVs. (A) Fraction of rare indels in coding-gene categories. Total number of indels shown. (B) Enrichment of SVs affecting functional annotations. Middle box shows genes, pseudogenes, and TF motifs; upper blow-out shows gene parts in different modes, and bottom blow-out shows enhancers with different formation mechanisms, i.e., NAHR, NH (nonhomologous), TEI (transposable element insertion), and VNTR (variable number of tandem repeats). Asterisks indicate significant enrichment (green) or depletion (red) after multiple hypothesis correction. SVs intersecting various functional categories in different modes (e.g., whole/partial) are shown in the right-hand schematics. (C) Aggregation of histone signal around breakpoints of deletions formed by different mechanisms. Breakpoints centered at zero. Aggregation for upstream and downstream regions corresponds to negative and positive distance, respectively. Signals for an activating histone mark (H3K4me1) and a repressive mark (H3K27me3) are shown.

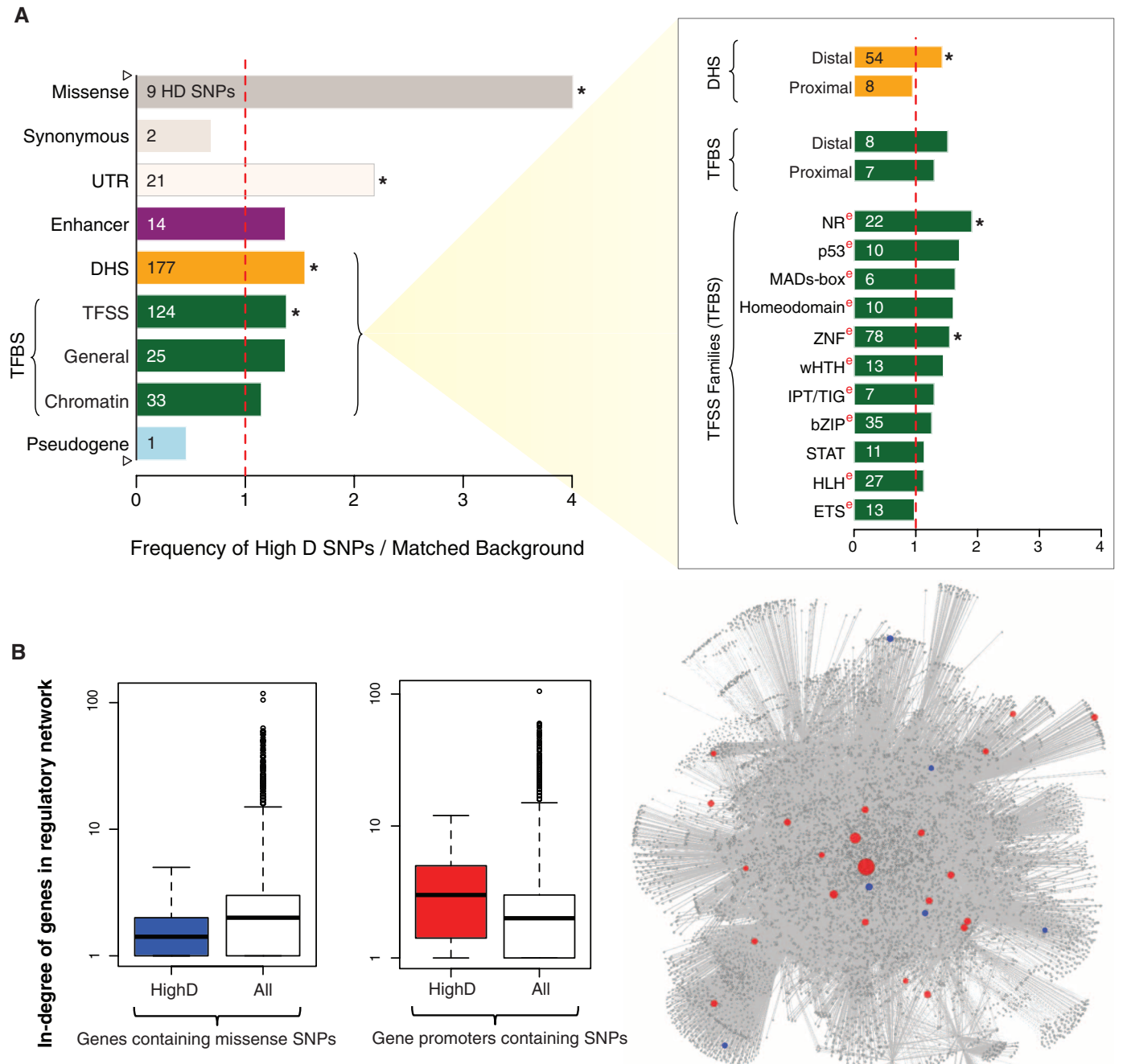
Another feature of somatic mutations associated with their potential role as drivers is their recurrence in the same genomic element across multiple cancer samples. We found that some non-coding elements from our functional categories show recurrent mutations (fig. S23). For example, the pseudogene *RP5-857K21.6* is mutated in three

out of seven prostate cancer samples, and the promoter of *RPI* is mutated in two (17).

FunSeq: Tool for Identification of Candidate Drivers in Tumor Genomes

On the basis of the integrative analysis above, we developed a tool to filter somatic variants from

tumor genomes and obtain a short list of candidate driver mutations (funseq.gersteinlab.org). FunSeq first filters mutations overlapping 1000 Genomes variants and then prioritizes those in regions under strong selection (sensitive and ultrasensitive), breaking TF motifs, and those associated with hubs. It can score the deleterious



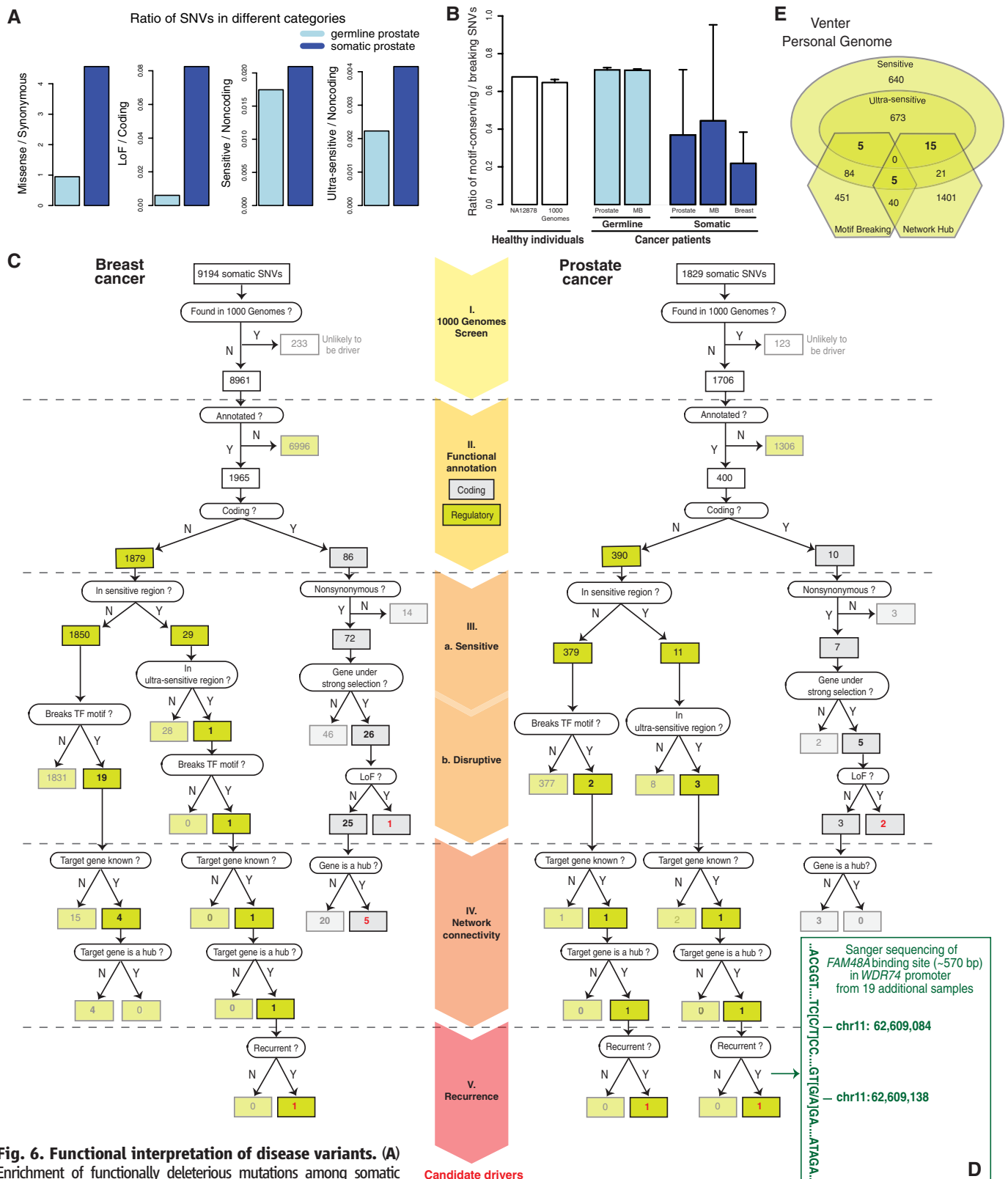


Fig. 6. Functional interpretation of disease variants. (A) Enrichment of functionally deleterious mutations among somatic compared with germline SNVs. Mean values from seven prostate cancer samples shown (variation shown in fig. S16). (B) Ratios for the number of SNVs that conserve versus break TF-binding motifs depicted for NA12878, the average of 1000 Genomes Phase I samples, and the average of somatic and germline samples from different cancers. Error bars represent 1 SD. MB, medulloblastoma. (C) Filtering of somatic variants from a breast (PD4006, left) and a prostate (PR-2832,

right) cancer sample leading to identification of candidate drivers. (D) A part of the *FAM48A* binding site sequenced by Sanger sequencing in an independent cohort of 19 prostate cancer samples shown in green (with the coordinates of mutations observed in one sample). (E) Application of variants filtering scheme to Venter personal genome. Number of SNVs in various categories shown.

potential of variants in single or multiple genomes and output the results in easy-to-use formats (i.e., “decorated” variant call format files, fig. S29 and data S6). The scores for each noncoding variant vary from 0 to 6, with 6 corresponding to maximum deleterious effect. When multiple tumor genomes are given as input, FunSeq also identifies recurrent mutations in the same element. Although our emphasis is on noncoding variants, it also outputs scores for coding variants.

We demonstrate the application of FunSeq as a workflow on representative breast and prostate cancer genomes (Fig. 6C). In the breast cancer sample, the workflow yielded one noncoding SNV likely to have strong phenotypic consequences: This SNV (i) occurs in an ultrasensitive region (*BRF2* binding site); (ii) breaks a *PAX-5* TF binding motif; (iii) is associated with a network hub (44); and (iv) is recurrent—that is, the regulatory module contains somatic mutations in multiple breast-cancer samples. In a similar fashion, the prostate-cancer sample revealed two noncoding SNVs predicted to have strong functional consequences (Fig. 6C). One of these is in an ultrasensitive region (*FAM48A* binding site) and lies in the promoter of *WDR74* gene (a hub in the PPI network with degree centrality = 56). We further tested the presence of mutations in this binding site by polymerase chain reaction followed by Sanger sequencing in an independent cohort of 19 prostate-cancer samples (45). We found that one sample in the cohort also harbors mutations in this region (Fig. 6D and fig. S25). Furthermore, we also observed increased expression of *WDR74* in the tumor relative to benign samples (fig. S26). These experimental results provide support for a likely functional role of this candidate driver.

A large-scale application of our tool to three medulloblastoma, 21 breast, and 64 prostate cancer genomes provided a total of 98 noncoding candidate drivers (table S8 and data S6) (17–20). Among these candidates, 68 occur in sensitive regions, 55 break TF motifs, and 90 target network hubs.

Generalized Identification of Deleterious Variants in Personal Genomes

Although we envision the most effective use of our tool for tumor genomes, it can also be applied to germline sequences to identify potentially deleterious variants. We applied it to four personal genomes: Snyder, Venter, NA12878, and NA19240 (46–48). Out of ~3 million SNVs, we were able to identify ~15 (range from 6 to 26) noncoding SNVs per individual with high scores from FunSeq (>4), indicating their potential deleterious effects (Fig. 6E, tables S9 and S10, and data S6 and S7). Thus, our approach can be used to prioritize noncoding variants in personal genomes as well.

Discussion

We identified the sensitive and ultrasensitive noncoding elements, which exhibit depletion of com-

mon polymorphisms and strong enrichment of known, inherited disease-causing mutations. Because they cover a small fraction of the entire genome (comparable to the exome), these regions can be probed alongside exome sequences in clinical studies. We found that functionally disruptive noncoding mutations tend to be under strong selection: In an analogous manner to LoF variants in coding genes, variants that break motifs in TF binding sites are selected against. There is a close relation between connectivity in biological networks and selective constraints: Higher connectivity is generally associated with higher constraint. Furthermore, selection against indels and large SVs acts in a similar fashion as against SNPs overall; however, the large size of SVs sometimes leads to a complex relation with functional elements. On the basis of these patterns of negative selection in functional elements, we developed a workflow and a corresponding software tool to prioritize noncoding variants in disease studies.

The prioritization scheme presented in our paper can be readily extended by incorporation of genomic polymorphisms from larger populations and higher-resolution functional annotations. Moreover, with the availability of RNA-seq data from large cohorts, additional genomic features such as eQTLs can be folded in. Our approach can be immediately applied in precision medicine studies to prioritize noncoding variants for follow-up characterization, particularly candidate driver mutations in cancer, and it can be further extended in the future.

Materials and Methods

Details of all data sets and methods are provided in the supplementary materials. A brief summary of major data sets and methods is provided here. SNPs, indels, and SVs from 1000 Genomes Phase I release were used to investigate patterns of selection in DNA elements (24). Noncoding annotations were obtained from ENCODE Integrative paper release (2). Although we did analyze broad functional annotations, such as all TFBSs, we focused on highly specific categories such as distal binding sites of factor *ZNF274*. A randomization procedure, similar to the Genome Structure Correction (2), was developed by considering the dependency structure of different categories to deal with multiple hypothesis correction while identifying the categories under significantly strong selection. Patterns of somatic mutations were obtained from seven prostate cancer (17), three medulloblastoma (20), and 21 breast cancer genomes (19), whereas driver mutations were also identified in additional 57 prostate cancer genomes (18).

References and Notes

- B. Yngvadottir, D. G. MacArthur, H. Jin, C. Tyler-Smith, The promise and reality of personal genomics. *Genome Biol.* **10**, 237 (2009). doi: [10.1186/gb-2009-10-9-237](https://doi.org/10.1186/gb-2009-10-9-237); pmid: [19723346](https://pubmed.ncbi.nlm.nih.gov/19723346/)
- I. Dunham et al., An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012). doi: [10.1038/nature11247](https://doi.org/10.1038/nature11247); pmid: [22955616](https://pubmed.ncbi.nlm.nih.gov/22955616/)
- M. T. Maurano et al., Systematic localization of common disease-associated variation in regulatory DNA. *Science* **337**, 1190–1195 (2012); doi: [10.1126/science.1222794](https://doi.org/10.1126/science.1222794); pmid: [22955828](https://pubmed.ncbi.nlm.nih.gov/22955828/)
- L. D. Ward, M. Kellis, Interpreting noncoding genetic variation in complex traits and human disease. *Nat. Biotechnol.* **30**, 1095–1106 (2012). doi: [10.1038/nbt.2422](https://doi.org/10.1038/nbt.2422); pmid: [23138309](https://pubmed.ncbi.nlm.nih.gov/23138309/)
- A. Visel et al., Targeted deletion of the 9p21 non-coding coronary artery disease risk interval in mice. *Nature* **464**, 409–412 (2010). doi: [10.1038/nature08801](https://doi.org/10.1038/nature08801); pmid: [20173736](https://pubmed.ncbi.nlm.nih.gov/20173736/)
- W. Lee, P. Yue, Z. Zhang, Analytical methods for inferring functional effects of single base pair substitutions in human cancers. *Hum. Genet.* **126**, 481–498 (2009). doi: [10.1007/s00439-009-0677-y](https://doi.org/10.1007/s00439-009-0677-y); pmid: [19434427](https://pubmed.ncbi.nlm.nih.gov/19434427/)
- L. D. Ward, M. Kellis, Evidence of abundant purifying selection in humans for recently acquired regulatory functions. *Science* **337**, 1675–1678 (2012); doi: [10.1126/science.1225057](https://doi.org/10.1126/science.1225057); pmid: [22956687](https://pubmed.ncbi.nlm.nih.gov/22956687/)
- X. J. Mu, Z. J. Lu, Y. Kong, H. Y. Lam, M. B. Gerstein, Analysis of genomic variation in non-coding elements using population-scale sequencing data from the 1000 Genomes Project. *Nucleic Acids Res.* **39**, 7058–7076 (2011). doi: [10.1093/nar/gkr342](https://doi.org/10.1093/nar/gkr342); pmid: [21596777](https://pubmed.ncbi.nlm.nih.gov/21596777/)
- B. Vernot et al., Personal and population genomics of human regulatory variation. *Genome Res.* **22**, 1689–1697 (2012). doi: [10.1101/gr.134890.111](https://doi.org/10.1101/gr.134890.111); pmid: [22955981](https://pubmed.ncbi.nlm.nih.gov/22955981/)
- S. Horn et al., *TERT* promoter mutations in familial and sporadic melanoma. *Science* **339**, 959–961 (2013); doi: [10.1126/science.1230062](https://doi.org/10.1126/science.1230062); pmid: [23348503](https://pubmed.ncbi.nlm.nih.gov/23348503/)
- F. W. Huang et al., Highly recurrent *TERT* promoter mutations in human melanoma. *Science* **339**, 957–959 (2013); doi: [10.1126/science.1229259](https://doi.org/10.1126/science.1229259); pmid: [23348506](https://pubmed.ncbi.nlm.nih.gov/23348506/)
- P. J. Killela et al., *TERT* promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 6021–6026 (2013). doi: [10.1073/pnas.1303607110](https://doi.org/10.1073/pnas.1303607110); pmid: [23530248](https://pubmed.ncbi.nlm.nih.gov/23530248/)
- D. Bell et al., Integrated genomic analyses of ovarian carcinoma. *Nature* **474**, 609–615 (2011). doi: [10.1038/nature10166](https://doi.org/10.1038/nature10166); pmid: [21720365](https://pubmed.ncbi.nlm.nih.gov/21720365/)
- D. M. Muzny et al., Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337 (2012). doi: [10.1038/nature11252](https://doi.org/10.1038/nature11252); pmid: [22810696](https://pubmed.ncbi.nlm.nih.gov/22810696/)
- P. S. Hammerman et al., Comprehensive genomic characterization of squamous cell lung cancers. *Nature* **489**, 519–525 (2012). doi: [10.1038/nature11404](https://doi.org/10.1038/nature11404); pmid: [22960745](https://pubmed.ncbi.nlm.nih.gov/22960745/)
- T. J. Hudson et al., International network of cancer genome projects. *Nature* **464**, 993–998 (2010). doi: [10.1038/nature08987](https://doi.org/10.1038/nature08987); pmid: [20393554](https://pubmed.ncbi.nlm.nih.gov/20393554/)
- M. F. Berger et al., The genomic complexity of primary human prostate cancer. *Nature* **470**, 214–220 (2011). doi: [10.1038/nature09744](https://doi.org/10.1038/nature09744); pmid: [21307934](https://pubmed.ncbi.nlm.nih.gov/21307934/)
- S. C. Baca et al., Punctuated evolution of prostate cancer genomes. *Cell* **153**, 666–677 (2013). doi: [10.1016/j.cell.2013.03.021](https://doi.org/10.1016/j.cell.2013.03.021); pmid: [23622249](https://pubmed.ncbi.nlm.nih.gov/23622249/)
- S. Nik-Zainal et al., Mutational processes molding the genomes of 21 breast cancers. *Cell* **149**, 979–993 (2012). doi: [10.1016/j.cell.2012.04.024](https://doi.org/10.1016/j.cell.2012.04.024); pmid: [22608084](https://pubmed.ncbi.nlm.nih.gov/22608084/)
- T. Rausch et al., Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. *Cell* **148**, 59–71 (2012). doi: [10.1016/j.cell.2011.12.013](https://doi.org/10.1016/j.cell.2011.12.013); pmid: [22265402](https://pubmed.ncbi.nlm.nih.gov/22265402/)
- G. Bejerano et al., Ultraconserved elements in the human genome. *Science* **304**, 1321–1325 (2004); doi: [10.1126/science.1098119](https://doi.org/10.1126/science.1098119); pmid: [15131266](https://pubmed.ncbi.nlm.nih.gov/15131266/)
- R. E. Mills et al., Mapping copy number variation by population-scale genome sequencing. *Nature* **470**, 59–65 (2011). doi: [10.1038/nature09708](https://doi.org/10.1038/nature09708); pmid: [21293372](https://pubmed.ncbi.nlm.nih.gov/21293372/)

23. R. Redon *et al.*, Global variation in copy number in the human genome. *Nature* **444**, 444–454 (2006). doi: [10.1038/nature05329](https://doi.org/10.1038/nature05329); pmid: [17122850](https://pubmed.ncbi.nlm.nih.gov/17122850/)
24. G. R. Abecasis *et al.*, An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012). doi: [10.1038/nature11632](https://doi.org/10.1038/nature11632); pmid: [23128226](https://pubmed.ncbi.nlm.nih.gov/23128226/)
25. Z. D. Zhang, A. Frankish, T. Hunt, J. Harrow, M. Gerstein, Identification and analysis of unitary pseudogenes: Historic and contemporary gene losses in humans and other primates. *Genome Biol.* **11**, R26 (2010). doi: [10.1186/gb-2010-11-3-r26](https://doi.org/10.1186/gb-2010-11-3-r26); pmid: [20210993](https://pubmed.ncbi.nlm.nih.gov/20210993/)
26. P. D. Stenson *et al.*, The Human Gene Mutation Database: 2008 update. *Genome Med* **1**, 13 (2009). doi: [10.1186/gm13](https://doi.org/10.1186/gm13); pmid: [19348700](https://pubmed.ncbi.nlm.nih.gov/19348700/)
27. C. Solis, G. I. Aizencang, K. H. Astrin, D. F. Bishop, R. J. Desnick, Uroporphyrinogen III synthase erythroid promoter mutations in adjacent GATA1 and CP2 elements cause congenital erythropoietic porphyria. *J. Clin. Invest.* **107**, 753–762 (2001). doi: [10.1172/JCI10642](https://doi.org/10.1172/JCI10642); pmid: [11254675](https://pubmed.ncbi.nlm.nih.gov/11254675/)
28. P. Hermanns *et al.*, Consequences of mutations in the non-coding RMRP RNA in cartilage-hair hypoplasia. *Hum. Mol. Genet.* **14**, 3723–3740 (2005). doi: [10.1093/hmg/ddi403](https://doi.org/10.1093/hmg/ddi403); pmid: [16254002](https://pubmed.ncbi.nlm.nih.gov/16254002/)
29. M. B. Gerstein *et al.*, Architecture of the human regulatory network derived from ENCODE data. *Nature* **489**, 91–100 (2012). doi: [10.1038/nature11245](https://doi.org/10.1038/nature11245); pmid: [22955619](https://pubmed.ncbi.nlm.nih.gov/22955619/)
30. H. B. Fraser, A. E. Hirsh, L. M. Steinmetz, C. Scharfe, M. W. Feldman, Evolutionary rate in the protein interaction network. *Science* **296**, 750–752 (2002). doi: [10.1126/science.1068696](https://doi.org/10.1126/science.1068696); pmid: [11976460](https://pubmed.ncbi.nlm.nih.gov/11976460/)
31. E. Khurana, Y. Fu, J. Chen, M. Gerstein, Interpretation of genomic variants using a unified biological network approach. *PLOS Comput. Biol.* **9**, e1002886 (2013). doi: [10.1371/journal.pcbi.1002886](https://doi.org/10.1371/journal.pcbi.1002886); pmid: [23505346](https://pubmed.ncbi.nlm.nih.gov/23505346/)
32. X. Wang *et al.*, Three-dimensional reconstruction of protein networks provides insight into human genetic disease. *Nat. Biotechnol.* **30**, 159–164 (2012). doi: [10.1038/nbt.2106](https://doi.org/10.1038/nbt.2106); pmid: [22252508](https://pubmed.ncbi.nlm.nih.gov/22252508/)
33. P. C. Sabeti *et al.*, Positive natural selection in the human lineage. *Science* **312**, 1614–1620 (2006). doi: [10.1126/science.1124309](https://doi.org/10.1126/science.1124309); pmid: [16778047](https://pubmed.ncbi.nlm.nih.gov/16778047/)
34. J. Ohashi *et al.*, Extended linkage disequilibrium surrounding the hemoglobin E variant due to malarial selection. *Am. J. Hum. Genet.* **74**, 1198–1208 (2004). doi: [10.1086/421330](https://doi.org/10.1086/421330); pmid: [15114532](https://pubmed.ncbi.nlm.nih.gov/15114532/)
35. M. T. Hamblin, A. Di Rienzo, Detection of the signature of natural selection in humans: Evidence from the Duffy blood group locus. *Am. J. Hum. Genet.* **66**, 1669–1679 (2000). doi: [10.1086/302879](https://doi.org/10.1086/302879); pmid: [10762551](https://pubmed.ncbi.nlm.nih.gov/10762551/)
36. L. B. Barreiro, G. Laval, H. Quach, E. Patin, L. Quintana-Murci, Natural selection has driven population differentiation in modern humans. *Nat. Genet.* **40**, 340–345 (2008). doi: [10.1038/ng.78](https://doi.org/10.1038/ng.78); pmid: [18246066](https://pubmed.ncbi.nlm.nih.gov/18246066/)
37. Y. Xue *et al.*, Population differentiation as an indicator of recent positive selection in humans: An empirical evaluation. *Genetics* **183**, 1065–1077 (2009). doi: [10.1534/genetics.109.107722](https://doi.org/10.1534/genetics.109.107722); pmid: [19737746](https://pubmed.ncbi.nlm.nih.gov/19737746/)
38. R. D. Hernandez *et al.*, Classic selective sweeps were rare in recent human evolution. *Science* **331**, 920–924 (2011). doi: [10.1126/science.1198878](https://doi.org/10.1126/science.1198878); pmid: [21330547](https://pubmed.ncbi.nlm.nih.gov/21330547/)
39. S. R. Grossman *et al.*, Identifying recent adaptations in large-scale genomic data. *Cell* **152**, 703–713 (2013). doi: [10.1016/j.cell.2013.01.035](https://doi.org/10.1016/j.cell.2013.01.035); pmid: [23415221](https://pubmed.ncbi.nlm.nih.gov/23415221/)
40. M. Akiyama *et al.*, Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J. Clin. Invest.* **115**, 1777–1784 (2005). doi: [10.1172/JCI24834](https://doi.org/10.1172/JCI24834); pmid: [16007253](https://pubmed.ncbi.nlm.nih.gov/16007253/)
41. P. M. Kim, J. O. Korbel, M. B. Gerstein, Positive selection at the protein network periphery: Evaluation in terms of structural constraints and cellular context. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 20274–20279 (2007). doi: [10.1073/pnas.0710183104](https://doi.org/10.1073/pnas.0710183104); pmid: [18077332](https://pubmed.ncbi.nlm.nih.gov/18077332/)
42. R. Haygood, O. Fedrigo, B. Hanson, K. D. Yokoyama, G. A. Wray, Promoter regions of many neural- and nutrition-related genes have experienced positive selection during human evolution. *Nat. Genet.* **39**, 1140–1144 (2007). doi: [10.1038/ng2104](https://doi.org/10.1038/ng2104); pmid: [17694055](https://pubmed.ncbi.nlm.nih.gov/17694055/)
43. S. B. Carroll, Evo-devo and an expanding evolutionary synthesis: A genetic theory of morphological evolution. *Cell* **134**, 25–36 (2008). doi: [10.1016/j.cell.2008.06.030](https://doi.org/10.1016/j.cell.2008.06.030); pmid: [18614008](https://pubmed.ncbi.nlm.nih.gov/18614008/)
44. K. Y. Yip *et al.*, Classification of human genomic regions based on experimentally determined binding sites of more than 100 transcription-related factors. *Genome Biol.* **13**, R48 (2012). doi: [10.1186/gb-2012-13-9-r48](https://doi.org/10.1186/gb-2012-13-9-r48); pmid: [22950945](https://pubmed.ncbi.nlm.nih.gov/22950945/)
45. C. E. Barbieri *et al.*, Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat. Genet.* **44**, 685–689 (2012). doi: [10.1038/ng.2279](https://doi.org/10.1038/ng.2279); pmid: [22610119](https://pubmed.ncbi.nlm.nih.gov/22610119/)
46. R. M. Durbin *et al.*, A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061–1073 (2010). doi: [10.1038/nature09534](https://doi.org/10.1038/nature09534); pmid: [20981092](https://pubmed.ncbi.nlm.nih.gov/20981092/)
47. R. Chen *et al.*, Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307 (2012). doi: [10.1016/j.cell.2012.02.009](https://doi.org/10.1016/j.cell.2012.02.009); pmid: [22424236](https://pubmed.ncbi.nlm.nih.gov/22424236/)
48. S. Levy *et al.*, The diploid genome sequence of an individual human. *PLoS Biol.* **5**, e254 (2007). doi: [10.1371/journal.pbio.0050254](https://doi.org/10.1371/journal.pbio.0050254); pmid: [17803354](https://pubmed.ncbi.nlm.nih.gov/17803354/)

Acknowledgments: We thank G. Boysen and C. O'Reilly for help with SNV experimental validation, K. Yip for target-gene identification, and Z. Liu for Web site design. T.H.P. is supported by the Danish Council for Independent Research Medical Sciences (FSS). Funding at the European Bioinformatics Institute is provided by European Molecular Biology Laboratory and the Wellcome Trust (WT085532 and WT095908). C.T.-S. acknowledges grant 098051 from the Wellcome-Trust Sanger Institute. Funding for the Institute for Precision Medicine (Weill Cornell Medical College/New York Presbyterian) is provided by National Cancer Institute (NCI) grant R01CA152057 (A.S., M.G., and M.A.R.) and Early Detection Research Network NCI U01 CA111275 (M.A.R.). M.A.R. also thanks the Prostate Cancer Foundation. M.G. also acknowledges grants HG005718 and HG007000. G.M. acknowledges National Human Genome Research Institute grants R01HG4719 and U01HG6513. H.Y. and S.M.L. are supported by NCI grant CA167824, National Institute of General Medical Sciences grant GM104424, and a Clinical and Translational Science Center Pilot Award and Cornell Seed Grant for intercampus collaborations. H.M.K., T.L., A.S., L.L., J.C., A.H., and J.D. contributed equally.

Supplementary Materials

www.sciencemag.org/content/342/6154/1235587/suppl/DC1
Materials and Methods
Supplementary Text
Fig. S1 to S29
Tables S1 to S12
References (49–90)
Data S1 to S7

24 January 2013; accepted 23 July 2013
[10.1126/science.1235587](https://doi.org/10.1126/science.1235587)



Mice Genetically Deficient in Vasopressin V1a and V1b Receptors Are Resistant to Jet Lag

Yoshiaki Yamaguchi *et al.*

Science **342**, 85 (2013);

DOI: 10.1126/science.1238599

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/85.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.85.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/85.full.html#related>

This article **cites 42 articles**, 14 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/85.full.html#ref-list-1>

This article has been **cited by 1** articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/342/6154/85.full.html#related-urls>

Mice Genetically Deficient in Vasopressin V1a and V1b Receptors Are Resistant to Jet Lag

Yoshiaki Yamaguchi,^{1*} Toru Suzuki,^{1*} Yasutaka Mizoro,¹ Hiroshi Kori,^{2,3} Kazuki Okada,¹ Yulin Chen,¹ Jean-Michel Fustin,¹ Fumiyoshi Yamazaki,¹ Naoki Mizuguchi,¹ Jing Zhang,⁴ Xin Dong,⁴ Gozoh Tsujimoto,⁵ Yasushi Okuno,⁶ Masao Doi,¹ Hitoshi Okamura^{1,4†}

Jet-lag symptoms arise from temporal misalignment between the internal circadian clock and external solar time. We found that circadian rhythms of behavior (locomotor activity), clock gene expression, and body temperature immediately reentrained to phase-shifted light-dark cycles in mice lacking vasopressin receptors V1a and V1b (*V1a^{-/-}V1b^{-/-}*). Nevertheless, the behavior of *V1a^{-/-}V1b^{-/-}* mice was still coupled to the internal clock, which oscillated normally under standard conditions. Experiments with suprachiasmatic nucleus (SCN) slices in culture suggested that interneuronal communication mediated by V1a and V1b confers on the SCN an intrinsic resistance to external perturbation. Pharmacological blockade of V1a and V1b in the SCN of wild-type mice resulted in accelerated recovery from jet lag, which highlights the potential of vasopressin signaling as a therapeutic target for management of circadian rhythm misalignment, such as jet lag and shift work.

The endogenous circadian clock drives oscillations in physiology and behavior with a period of about 24 hours. We are not usually aware of this system because it is completely synchronized with environmental light-dark cycles, but travelling rapidly across multiple time zones suddenly makes us aware of the desynchrony, causing sleep disturbances and gastrointestinal distress (1, 2). Repeated jet-lag exposure and rotating shift work increase the risk of lifestyle-related diseases, such as cardiovascular complaints and metabolic insufficiency (3, 4). Although jet lag is recognized as a chronobiological problem (5–7), its specific molecular and cellular mechanisms are poorly understood.

In mammals, internal time is orchestrated by the master clock in the hypothalamic suprachiasmatic nucleus (SCN), whose coherent output signal synchronizes cell clocks throughout the body (8–13). Hence, a misalignment of SCN clock signals and environmental time cues (e.g., light and temperature) would occur during jet lag. To identify candidate signaling molecules that might contribute to jet lag, we designed a screening strategy (14) in which we (i) used histochemistry to identify genes whose expression is enriched in the

mouse SCN, (ii) generated mutant mice lacking candidate genes of interest, and (iii) measured the locomotor activity of the mutant mice under experimental jet-lag conditions. Genes encoding brain peptides and their receptors were the initial candidates of interest, because many of them are expressed in the SCN (15). We identified arginine vasopressin (AVP) and its receptors (V1a and V1b receptors) as strong candidates. The circadian expression pattern of these proteins has been studied (16–18), but their role in the circadian clock is unclear, with the exception of the V1a receptor. Genetic deletion of this receptor causes period elongation in mice (19).

Locomotor Activities During Jet Lag

We generated V1a and V1b receptor double-knockout mice (*V1a^{-/-}V1b^{-/-}* mice) (20–22) and examined their behavioral rhythms under experimental jet-lag conditions. The mice were housed in a light (~200 lux)–controlled isolator with food and drink ad libitum, and their spontaneous locomotor activity was recorded by infrared sensor (Fig. 1, A to C) or voluntary wheel running (fig. S1). When they were maintained in a 12-hour light/12-hour dark (LD) cycle, both wild-type (WT) and *V1a^{-/-}V1b^{-/-}* mice exhibited high locomotor activity during the dark phase. After 2 weeks of recording behaviors, LD cycles were advanced by 8 hours. In WT mice, this advance evoked a gradual shift of locomotor activity rhythms, which took 8 to 10 days for complete reentrainment to the new LD schedule (Fig. 1, A and B). This slow resetting of locomotor activity rhythm—which is characterized by an activity onset that is not synchronous with “lights off” as normally observed, but is delayed into the night—was expected, as it is the typical sign that mice are experiencing jet lag. Every subsequent day after the LD cycle advance, the WT mice will start their activity slightly earlier, to

finally align, after 8 to 10 days, to the beginning of the night. In contrast, *V1a^{-/-}V1b^{-/-}* mice showed almost immediate reentrainment with only 2 to 4 days of transition (Fig. 1A).

For quantitative comparison between genotypes, we calculated the 50% phase-shift value (PS₅₀) (5) and found that *V1a^{-/-}V1b^{-/-}* mice reentrained more rapidly than WT mice (Fig. 1B, inset) ($P < 0.001$, unpaired *t* test). We then delayed LD cycles by 8 hours (Fig. 1, A and C) and found that, although WT mice required 5 to 6 days for reentrainment, *V1a^{-/-}V1b^{-/-}* mice required only 1 day (Fig. 1, A and C), and PS₅₀ was also much smaller in *V1a^{-/-}V1b^{-/-}* mice (Fig. 1C, inset) ($P < 0.001$, unpaired *t* test). We next examined which AVP receptor, V1a or V1b, dictates the rate of reentrainment after phase shift and found that single knockout (*V1a^{-/-}V1b^{+/+}* mice and *V1a^{+/+}V1b^{-/-}*) mice showed intermediate rates of reentrainment (figs. S2 and S3), which indicated that both receptors contribute to the accelerated reentrainment after jet lag.

Light has a dominant influence on the behavior of mice because they are nocturnal animals; it inhibits their locomotor activity or foraging behavior. To exclude the possibility that the locomotor activity of *V1a^{-/-}V1b^{-/-}* mice is merely caused by the ambient LD cycle, we released mice in constant darkness (DD) after a transient advance in LD cycles. After 4, 3, 2, and only 1 day advance in the LD cycle, larger phase advances in the free-running behavior of *V1a^{-/-}V1b^{-/-}* mice were observed compared with WT mice (Fig. 1, D to F, and fig. S4). *V1a^{-/-}V1b^{-/-}* mice also showed larger phase delays in behavior after only 1 day's delay in LD cycles (fig. S5). These findings strongly suggest that the immediate adaptation to a new LD cycle in *V1a^{-/-}V1b^{-/-}* mice is not a masking effect of the environmental LD cycle but a rapid phase shift of the endogenous clock. Of note, period length in DD conditions, phase angle from LD to DD, phase response curve obtained with a short light exposure, and expression of canonical clock genes (*Per1*, *Per2*, *Bmal1*, and *Dbp*) in *V1a^{-/-}V1b^{-/-}* SCN were virtually identical to those in WT mice (Fig. 1, G to J, and figs. S6 and S7). These behavioral and molecular data exclude the possibility that rapid reentrainment in *V1a^{-/-}V1b^{-/-}* mice is simply caused by a disabled clock. When behavioral experiments were performed under conditions of intermediate light intensity (~50 lux), we also found that *V1a^{-/-}V1b^{-/-}* mice reentrained faster after a phase advance or phase delay (figs. S8 and S9) and showed larger phase advances after a 1- to 4-day advance in LD cycles (fig. S10). Moreover, there were no significant differences between genotypes in the phase delay in locomotor activities, the level of *Per1* induction, or the number of *Per1*-positive cells induced by a light pulse at CT14 (CT represents circadian time; CT0 is subjective dawn and CT12 is subjective dusk) (fig. S11), which suggested that the light response of *V1a^{-/-}V1b^{-/-}* SCN clock is similar to that of WT.

¹Department of Systems Biology, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan. ²Department of Information Sciences, Ochanomizu University, Tokyo 112-8620, Japan. ³CREST, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan. ⁴Division of Molecular Brain Science, Department of Brain Science, Kobe University Graduate School of Medicine, Chuo-ku, Kobe 650-0017, Japan. ⁵Department of Genomic Drug Discovery Science, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan. ⁶Department of Systems Biosciences for Drug Discovery, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan.

*These authors contributed equally to this work.

†Corresponding author. E-mail: okamurah@pharm.kyoto-u.ac.jp

Oscillations of Clock Genes During Jet Lag

To characterize molecular changes in the SCN during jet lag, we examined clock gene expression in laser-microdissected SCN samples collected from mice killed every 4 hours (fig.

S12). Before a phase advance in LD cycles, all transcripts examined in both genotypes showed a similar expression pattern: *Per1*, *Per2*, and *Dbp* showed comparable peak values around the middle of the day, ZT6 to ZT10 (ZT represents

Zeitgeber time used in LD cycle; ZT0 is lights-on and ZT12 is lights-off), whereas *Bmal1* was highest around ZT14 (Fig. 2). In contrast, after a phase advance in LD cycles, there were striking differences between genotypes in clock gene

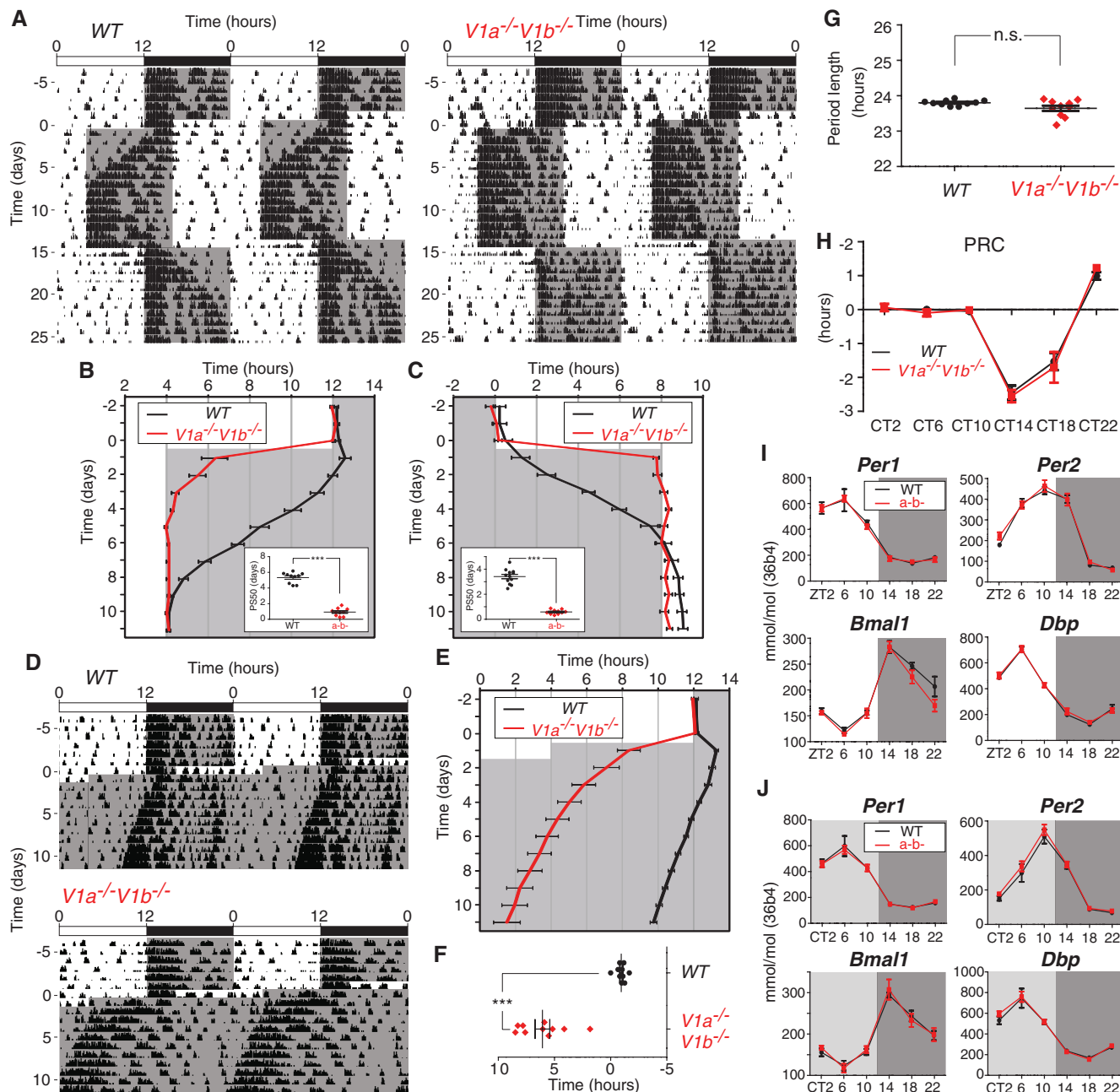


Fig. 1. *V1a^{-/-}V1b^{-/-}* mice subjected to an experimental jet-lag paradigm show immediate reentrainment to a new light-dark cycle. (A) Representative double-plotted actogram of WT (left) and *V1a^{-/-}V1b^{-/-}* (right) mice subjected to an 8-hour phase advance and delay in LD cycles. (B) Activity onset in the 8-hour phase advance [means $\pm$ SEM; $n = 10$ (both genotypes)]. (C) Activity offset in 8-hour phase delay [means $\pm$ SEM; $n = 12$ (WT) and 10 (*V1a^{-/-}V1b^{-/-}*)]. Insets (B and C) indicate PS₅₀ values in phase advance and in phase delay (means $\pm$ SEM; *** $P < 0.001$, unpaired t test). (D to F) Representative double-plotted actograms (D) of WT (top) and *V1a^{-/-}V1b^{-/-}* mice (bottom) subjected to an 8-hour phase advance on day 1 and released to DD, activity onset (E) and magnitude of the phase advance (F) [means $\pm$ SEM; $n = 10$ (both genotypes); *** $P < 0.001$, unpaired t test]. (G) Period length of WT and *V1a^{-/-}V1b^{-/-}* mice, based on a 14-day interval taken after 3 days of a

DD regime. Plotted are the period lengths of individual animals [means $\pm$ SEM; $n = 10$ (both genotypes); $P = 0.9524$, unpaired t test]. (H) Quantification of the magnitude of phase shifts by a brief light exposure (30 min of ~ 200 lux fluorescent light at CT2, 6, 10, 14, 18, and 22; $n = 8$ for both genotypes). By convention, delays are negative and advances are positive. Note that WT and *V1a^{-/-}V1b^{-/-}* mice have virtually the same phase-shift magnitude at all time points. (I and J) Clock gene expression in the SCN in LD (I) and in DD (J) conditions. SCN was dissected out by laser microdissection (see fig. S8), and each transcript was measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) [means $\pm$ SEM; $n = 5$ (both genotypes in both LD and DD)]. For this and subsequent figures, white and gray background indicates lights on and off, respectively. Black and red colors indicate WT and *V1a^{-/-}V1b^{-/-}*, respectively. Top bars indicate initial LD cycle.

expression. In WT mice, *Per1* and *Per2* expression lost rhythmicity on days 1 to 2, oscillated with very low amplitude on days 3 to 7, and recovered to the original circadian expression pattern on day 8. *Bmal1* and *Dbp* expression also required 8 to 9 days for complete recovery to the original circadian expression pattern, with severely perturbed rhythmicity on days 1 to 5 (Fig. 2). In *V1a⁺V1b⁺* mice, although the expression of some genes was partially perturbed on days 1 to 2, the peak time and amplitude in oscillations of all four clock genes recovered on day 3 (Fig. 2). Consistent with these results, cellular level analysis by digoxigenin in situ hybridization revealed that the robust oscillation in *Per1* and *Dbp* in WT SCN was severely perturbed on day 3 after a phase advance in LD cycles but not in *V1a⁺V1b⁺* SCN (fig. S13).

We next examined the phase-transition kinetics of clock gene rhythms in peripheral organs and monitored temperature rhythms. In contrast to

what we observed with the SCN, clock gene rhythm amplitudes in the liver were maintained during reentrainment. However, the peak time was severely affected after a phase advance in LD cycles: phase recovery required 9 to 10 days in WT mice but only 5 days in *V1a⁺V1b⁺* mice (Fig. 3, A and B). Similar reentrainment kinetics was also observed in the kidney (fig. S14). Body temperature rhythms peaking at ZT16 reentrained within 5 days in *V1a⁺V1b⁺* mice, but needed 10 days in WT mice after a phase advance in LD cycles (Fig. 3, C and D). These results demonstrate the rapid reentrainment of clock gene and body temperature rhythms in *V1a⁺V1b⁺* mice (see supplementary text for comparison with previous jet-lag studies in WT mice). In addition, reestablishment of the central SCN clock occurred 1 to 2 days earlier than that of the peripheral clock or body temperature rhythms in both genotypes, which suggests that the SCN clock determines the recovery period of the whole body.

Vasopressin Signaling and Interneuronal Communication in the SCN

It is well established that AVP neurons constitute the main population of the SCN and that AVP synthesis and release from the SCN to the cerebrospinal fluid exhibit a robust circadian rhythm (16, 23). AVP neurons mutually connect via synaptic contacts and form a local circuit within the SCN (24). *V1a* and *V1b* are expressed in SCN neurons (25), and our double in situ hybridization results revealed that most AVP neurons also expressed *V1a* in the dorsomedial SCN (fig. S15, A to D), which indicated abundant interactions between AVP neurons. Moreover, transcripts of *AVP* and *V1a* showed robust antiphasic rhythms (fig. S15, E and F), which suggested dynamic circadian regulation of ligand-receptor interactions (12).

We hypothesized that the disruption of clock gene rhythms observed in WT SCN, but not in *V1a⁺V1b⁺* SCN, during jet lag might be due to

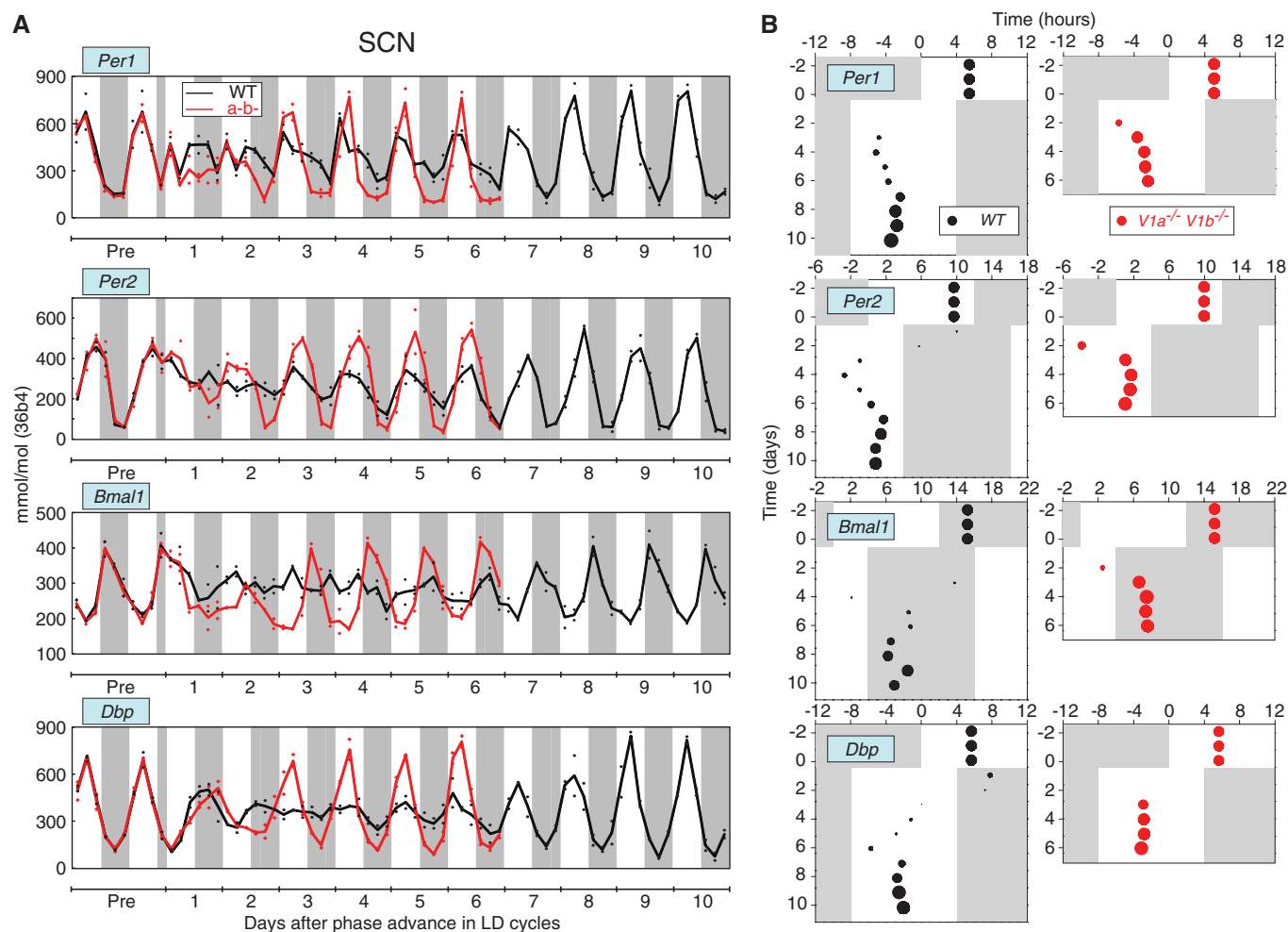


Fig. 2. *V1a⁺V1b⁺* mice subjected to an experimental jet-lag paradigm show fast reentrainment of SCN clock gene rhythms. (A) Measurement by qRT-PCR of clock gene expression profiles (*Per1*, *Per2*, *Bmal1*, and *Dbp*) in the laser microdissected SCN (fig. S8A), obtained every 4 hours continuously throughout the jet-lag schedule (fig. S8B). LD cycles were advanced by 8 hours on day 1. Graph indicates an average of two mice independently collected and measured. Note that all transcripts examined in WT SCN lost

rhythmicity right after jet lag. (B) Peak time- and amplitude-based kinetic analyses of reentrainment in clock gene expression rhythms. Peak time and amplitude are shown schematically as dots, position and diameter representing peak time and daily amplitude, respectively. Dots were omitted when daily peak time was ambiguous. Dots before an 8-hour phase advance are triple-plotted for better visualization. Black and red colors indicate WT and *V1a⁺V1b⁺*, respectively.

alterations in AVP-mediated interneuronal communication in the SCN. To investigate the effect of AVP-mediated communication on cellular rhythms, we performed real-time bioluminescence imaging of SCN slices from WT and $V1a^{-/-}V1b^{-/-}$ mice carrying *Per1*-promoter-luciferase (*Per1-luc*), which allows simultaneous measurement of hundreds of cellular rhythms (8). Both WT and $V1a^{-/-}V1b^{-/-}$ SCN neurons showed robust rhythms in a spatiotemporally

organized manner, starting from the dorsomedial portion and spreading to the ventrolateral side (Fig. 4 and movies S1 and S2). This phase order was perturbed by administration of cycloheximide (CHX), which resets the phase of all neurons (8). After CHX withdrawal, the original phase order among WT neurons recovered, whereas that among $V1a^{-/-}V1b^{-/-}$ neurons was severely permuted (Fig. 4, fig. S16, and Movie S3). These results indicate

that $V1a^{-/-}V1b^{-/-}$ SCN is more amenable to perturbation than WT SCN. Conceivably, it is this vulnerability in interneuronal coupling that enables the mutant SCN to reentrain to altered lighting schedules more rapidly.

To evaluate the effect of AVP-mediated cellular interaction in the SCN under jet-lag conditions, we built a mathematical model consisting of three oscillators in the SCN and one in the

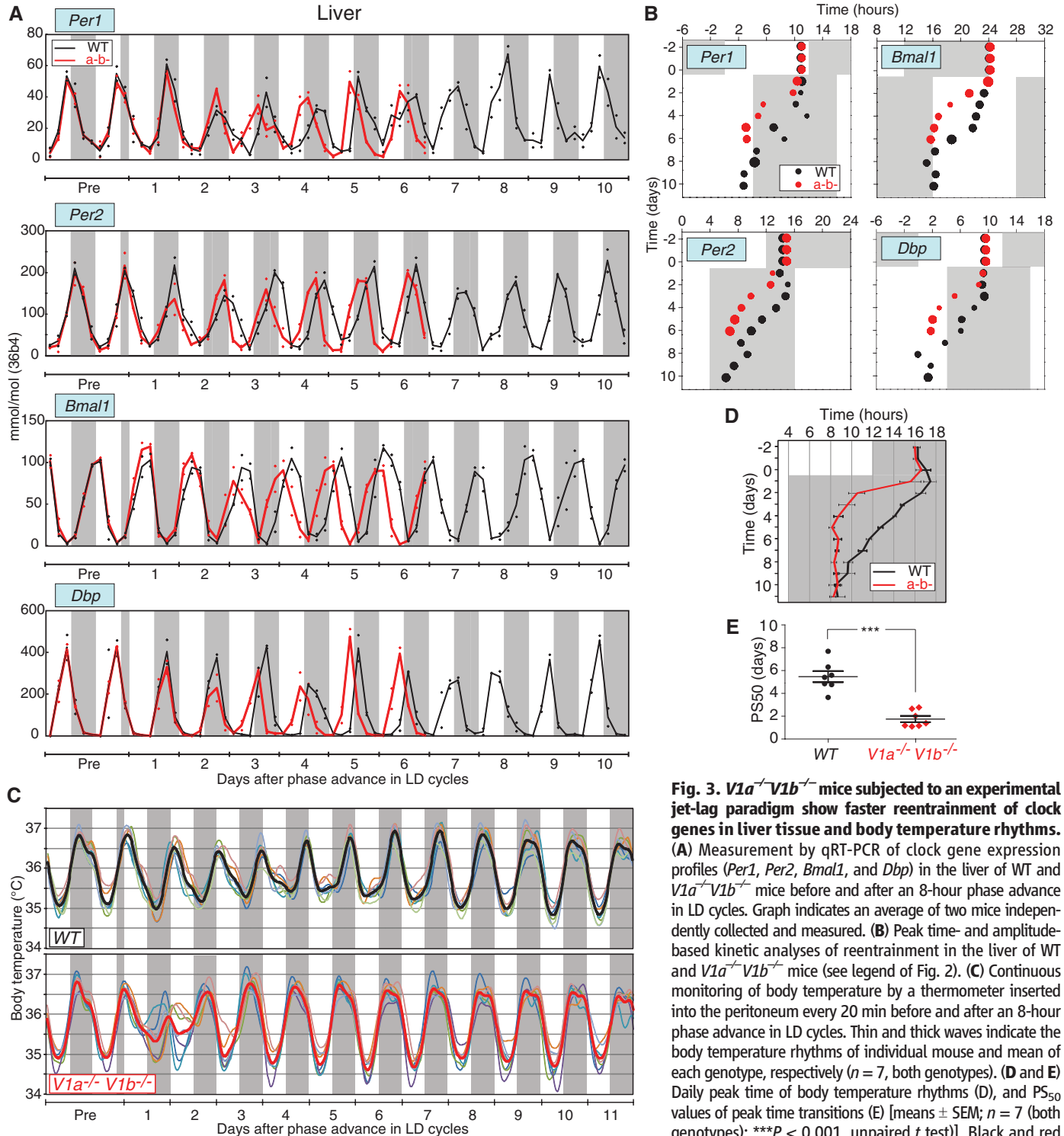


Fig. 3. $V1a^{-/-}V1b^{-/-}$ mice subjected to an experimental jet-lag paradigm show faster reentrainment of clock genes in liver tissue and body temperature rhythms. (A) Measurement by qRT-PCR of clock gene expression profiles (*Per1*, *Per2*, *Bmal1*, and *Dbp*) in the liver of WT and $V1a^{-/-}V1b^{-/-}$ mice before and after an 8-hour phase advance in LD cycles. Graph indicates an average of two mice independently collected and measured. (B) Peak time- and amplitude-based kinetic analyses of reentrainment in the liver of WT and $V1a^{-/-}V1b^{-/-}$ mice (see legend of Fig. 2). (C) Continuous monitoring of body temperature by a thermometer inserted into the peritoneum every 20 min before and after an 8-hour phase advance in LD cycles. Thin and thick waves indicate the body temperature rhythms of individual mouse and mean of each genotype, respectively ($n = 7$, both genotypes). (D and E) Daily peak time of body temperature rhythms (D), and PS50 values of peak time transitions (E) [means $\pm$ SEM; $n = 7$ (both genotypes); *** $P < 0.001$, unpaired t test]. Black and red colors indicate WT and $V1a^{-/-}V1b^{-/-}$, respectively.

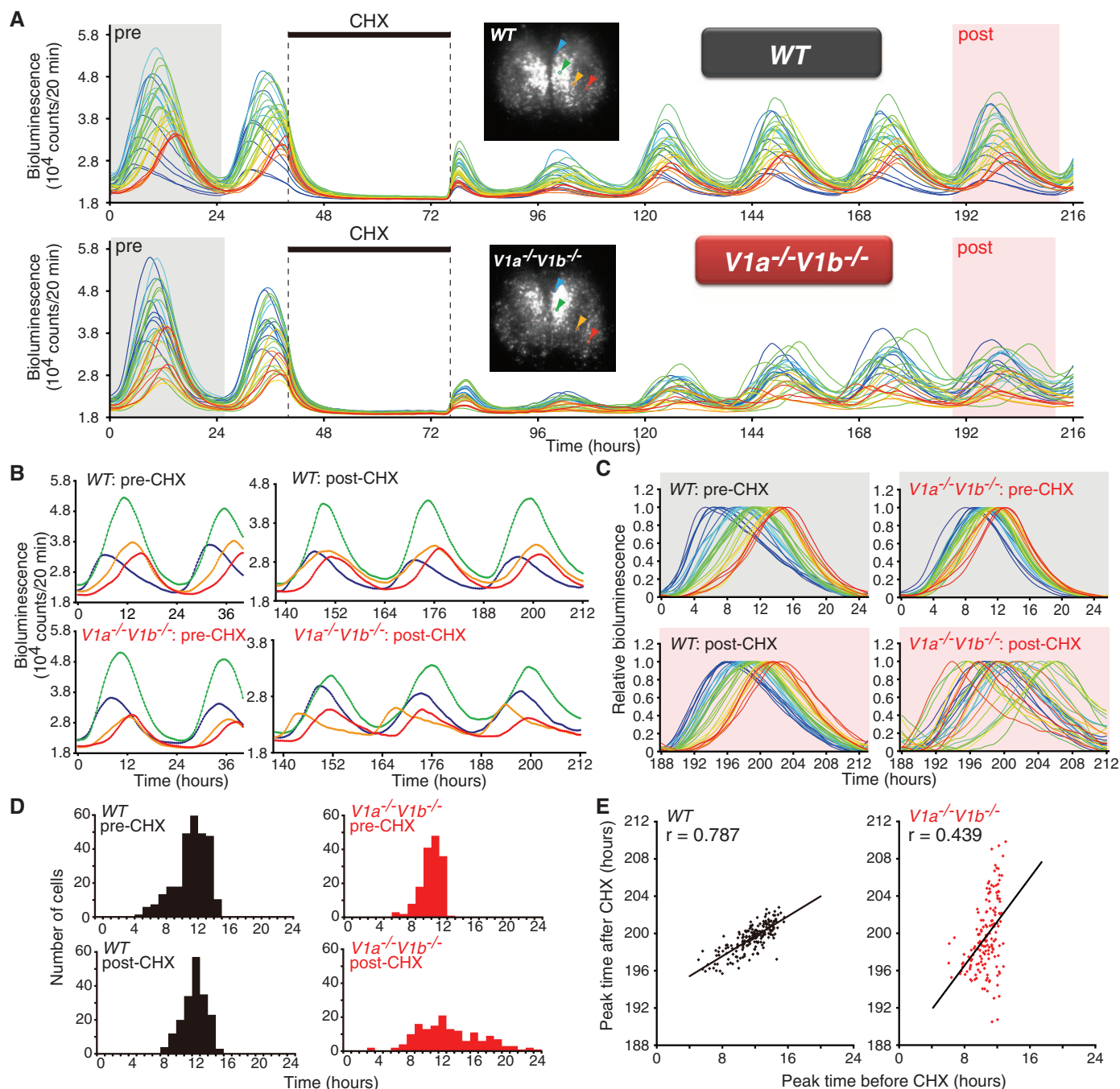


Fig. 4. Perturbation experiments with CHX reveal a weak spatiotemporal phase order organization in the SCN of $V1a^{-/-}V1b^{-/-}$ mice. (A) Representative cellular rhythms randomly chosen over the SCN slice (29 cells, both genotypes). Cellular oscillations showed a stable phase order in each cycle, but all cell clocks were reset with CHX application, and the first peaks of cells appeared simultaneously at 3 hours after CHX washout in both WT and $V1a^{-/-}V1b^{-/-}$ SCN. The phase order among the individual cells gradually recovered cycle by cycle in WT, whereas phases of cellular oscillations were severely permuted in $V1a^{-/-}V1b^{-/-}$ SCN. (B) Recording of bioluminescence of four representative cells pre- (0 to 40 hours) and post- (138 to 212 hours) CHX treatment [see inset photos in (A) for localization of cells; scale bar, 100 μ m]. Note the phase order of four cells (blue-green-orange-red) was maintained before and after CHX treatment in WT SCN, but it was not in

$V1a^{-/-}V1b^{-/-}$ SCN. (C) Normalized bioluminescence of individual cells during pre- [0 to 24 hours in (A), gray background] and post- [188 to 212 hours in (A), pink background] CHX treatment. Peak and trough values were adjusted to 1 and 0, respectively. (D) The range of peak times of randomly chosen cells [$n = 180$ (WT) and 155 ($V1a^{-/-}V1b^{-/-}$)] before and after CHX treatment. The x axis represents circadian hours (1 hour = peak interval/24 hours) from the trough time of the total SCN bioluminescence. (E) Correlation of the peak times of the individual cells [$n = 180$ (WT) and 155 ($V1a^{-/-}V1b^{-/-}$)] before CHX application (0 to 24 hours) with those after CHX washout (188 to 212 hours). Strong correlation in peak times before and after CHX treatment was observed in WT SCN, but not in $V1a^{-/-}V1b^{-/-}$ SCN. The results shown are representative of four independent experiments that yielded similar results.

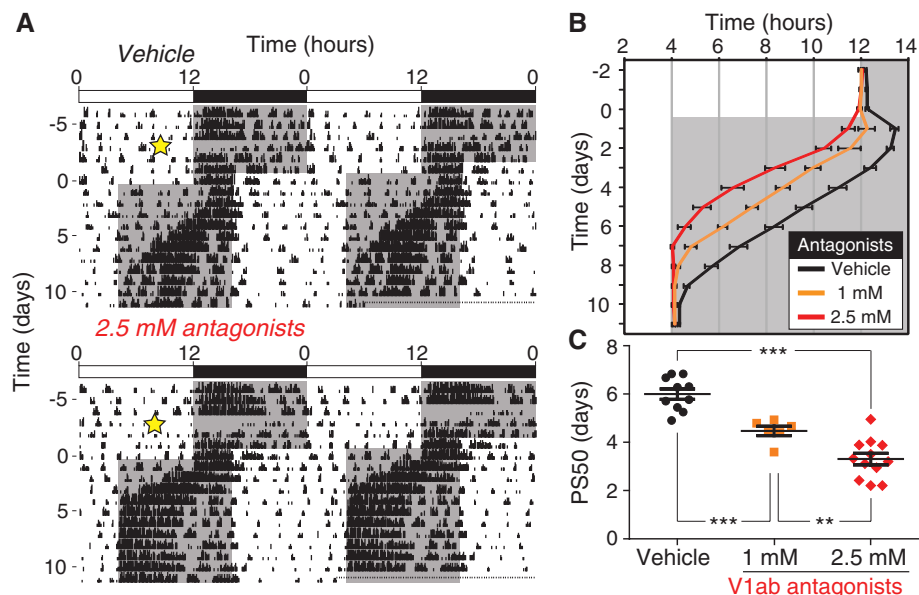


Fig. 5. Direct infusion of V1a and V1b antagonists into the SCN enhances the speed of reentrainment of WT mice subjected to an experimental jet-lag paradigm. (A to C) A mixture of OPC-21268 (V1a antagonist) and SSR 149415 (V1b antagonist) was applied over the SCN by osmotic minipump, and LD cycles were advanced by 8 hours on day 1. Two concentrations of each antagonist (1 mM or 2.5 mM) were applied. (A) Representative double-plotted actograms of vehicle- (top) or 2.5 mM antagonist-treated mice (bottom). Time of surgery is indicated by a yellow star. (B) Activity onset [means $\pm$ SEM; $n = 10, 6$, and 12 in vehicle, 1 mM, and 2.5 mM groups, respectively]. (C) PS₅₀ values of onset time transitions (means $\pm$ SEM). One-way analysis of variance (ANOVA), $F_{2,25} = 39.09$; $P < 0.0001$. Tukey-Kramer post hoc test was performed for comparison among the groups. ** $P < 0.01$, *** $P < 0.001$.

periphery. This model is based on a phase oscillator (26, 27) and is useful for understanding the dynamics of coupled multiple oscillators (see fig. S17 and supplementary text for details). This model showed that the WT SCN output lost rhythmicity for several days, whereas that of $V1a^{-/-}V1b^{-/-}$ recovered quickly after a phase advance in LD cycles (fig. S17, C and D). Faster reentrainment of peripheral output in $V1a^{-/-}V1b^{-/-}$ compared with WT was also recreated in our model (fig. S17, E and F).

Pharmacological Inhibition of V1a/V1b Signaling

Our mathematical model predicted that the weaker the AVP-mediated intercellular communication becomes, the shorter the reentrainment period (fig. S18). To test this experimentally, we transiently blocked V1a and V1b signaling in the SCN of adult WT mice in vivo during jet lag. We applied a mixture of OPC-21268 (V1a antagonist) and SSR 149415 (V1b antagonist) just over the SCN through an osmotic minipump (28). Mice receiving this treatment reentrained significantly faster to new LD cycles in a dose-dependent manner (Fig. 5). To examine the effect of these antagonists on the assembly of cellular rhythms among SCN neurons, we treated WT SCN slices with a mixture of antagonists after CHX application and found that the original phase order among WT SCN neurons was severely permuted as observed in $V1a^{-/-}V1b^{-/-}$ SCN (fig. S19). These results support the crucial role of V1a and V1b signaling in the SCN in determining the speed of reentrainment after a phase advance in LD cycles and exclude the possibility that

rapid reentrainment in $V1a^{-/-}V1b^{-/-}$ mice is simply caused by a developmental failure in mutant mice.

Conclusions

In summary, we have shown that mice lacking the vasopressin V1a and V1b receptors are resistant to jet lag, as measured by locomotor activity, clock gene expressions, and body temperature. The mutant mice rapidly phase-shift to a new environmental LD cycle. Like AVP and its receptors, vasoactive intestinal polypeptide and its receptor VPAC2 are also expressed in the SCN and have been linked to the circadian clock. However, deficiency of VPAC2 in mice has severe consequences on behavior and neural activity rhythms, as well as clock gene oscillations (10, 29). In contrast, we showed that the absence of V1a and V1b receptors does not elicit overt anomalies in behavioral and clock gene expression rhythms under regular LD and constant DD conditions. Previous work suggested that genetic manipulation of AVP or its receptor have only minor effects on circadian behavior (19, 30). Here, however, we demonstrated that rhythms of behavior, clock gene oscillation, and body temperature in $V1a^{-/-}V1b^{-/-}$ mice reentrain immediately after a phase advance in LD cycles. We hypothesize that this occurs because AVP-mediated interneuronal communication, which confers on the SCN an intrinsic resistance to external perturbations like jet lag, is missing in $V1a^{-/-}V1b^{-/-}$ mice.

Epidemiological studies have shown that chronic jet lag and rotating shift work can increase an

individual's risk of developing hypertension, obesity, and other metabolic disorders. Our results identify vasopressin signaling as a possible therapeutic target for the management of circadian rhythm misalignment.

References and Notes

1. C. A. Comperatore, G. P. Krueger, *Occup. Med.* **5**, 323–341 (1990).
2. R. L. Sack, *Travel Med. Infect. Dis.* **7**, 102–110 (2009).
3. O. M. Buxton *et al.*, *Sci. Transl. Med.* **4**, 129ra43 (2012).
4. F. A. Scheer, M. F. Hilton, C. S. Mantzoros, S. A. Shea, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4453–4458 (2009).
5. S. Kiessling, G. Eichele, H. Oster, *J. Clin. Invest.* **120**, 2600–2609 (2010).
6. S. Yamazaki *et al.*, *Science* **288**, 682–685 (2000).
7. A. B. Reddy, M. D. Field, E. S. Maywood, M. H. Hastings, *J. Neurosci.* **22**, 7326–7330 (2002).
8. S. Yamaguchi *et al.*, *Science* **302**, 1408–1412 (2003).
9. J. A. Mohawk, J. S. Takahashi, *Trends Neurosci.* **34**, 349–358 (2011).
10. S. J. Aton, C. S. Colwell, A. J. Harmar, J. Waschek, E. D. Herzog, *Nat. Neurosci.* **8**, 476–483 (2005).
11. M. P. Butler, R. Silver, *J. Biol. Rhythms* **24**, 340–352 (2009).
12. E. S. Maywood, J. E. Chesham, J. A. O'Brien, M. H. Hastings, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14306–14311 (2011).
13. R. Cao, G. Q. Butcher, K. Karelina, J. S. Arthur, K. Obrietan, *Eur. J. Neurosci.* **37**, 130–140 (2013).
14. H. Okamura, *Cold Spring Harb. Symp. Quant. Biol.* **72**, 551–556 (2007).
15. A. N. van den Pol, K. L. Tsujimoto, *Neuroscience* **15**, 1049–1086 (1985).
16. W. J. Schwartz, S. M. Reppert, *J. Neurosci.* **5**, 2771–2778 (1985).
17. D. F. Swaab, C. W. Pool, F. Nijveldt, *J. Neural Transm.* **36**, 195–215 (1975).
18. W. S. Young 3rd, K. Kovács, S. J. Lolait, *Endocrinology* **133**, 585–590 (1993).
19. J. D. Li, K. J. Burton, C. Zhang, S. B. Hu, Q. Y. Zhou, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296**, R824–R830 (2009).
20. T. A. Koshimizu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 7807–7812 (2006).
21. A. Tanoue *et al.*, *J. Clin. Invest.* **113**, 302–309 (2004).
22. Materials and methods are available as supplementary material on Science Online.
23. X. Jin *et al.*, *Cell* **96**, 57–68 (1999).
24. M. Castel, N. Feinstein, S. Cohen, N. Harari, *J. Comp. Neurol.* **298**, 172–187 (1990).
25. T. Kalamatianos, I. Kalló, C. W. Coen, *J. Neuroendocrinol.* **16**, 493–501 (2004).
26. A. T. Winfree, *J. Theor. Biol.* **16**, 15–42 (1967).
27. Y. Kuramoto, *Chemical Oscillations, Waves, and Turbulence* (Dover Publications, New York, 2003).
28. M. Doi *et al.*, *Nat. Commun.* **2**, 327 (2011).
29. A. J. Harmar *et al.*, *Cell* **109**, 497–508 (2002).
30. T. A. Groblewski, A. A. Nunez, R. M. Gold, *Brain Res. Bull.* **6**, 125–130 (1981).

Acknowledgments: This work was supported in part by Grant-in-Aid for Specially Promoted Research (to H.O.) and Scientific Research on Innovative Areas “Brain Environment” (to H.O.) from the Ministry of Education, Science, Sports and Culture of Japan, and by grants from Takeda Science Foundation (to H.O.), SRF (to H.O.), and Kanoe Science Foundation (to Y.Y.). H.O. and Kyoto University have applied for a patent (PCT/JP2013/064376) related to the use of V1a and V1b antagonists as circadian rhythm modulators.

Supplementary Materials

www.sciencemag.org/content/342/6154/85/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S19
References (31–44)
Movies S1 to S3

1 April 2013; accepted 22 August 2013
10.1126/science.1238599



Selective Gas Transport Through Few-Layered Graphene and Graphene Oxide Membranes

Hyo Won Kim *et al.*

Science **342**, 91 (2013);

DOI: 10.1126/science.1236098

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/91.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.91.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/91.full.html#related>

This article **cites 30 articles**, 5 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/91.full.html#ref-list-1>

Selective Gas Transport Through Few-Layered Graphene and Graphene Oxide Membranes

Hyo Won Kim,^{1*} Hee Wook Yoon,^{1*} Seon-Mi Yoon,^{2,3*} Byung Min Yoo,¹ Byung Kook Ahn,¹ Young Hoon Cho,¹ Hye Jin Shin,¹ Hoichang Yang,⁴ Ungyu Paik,¹ Soongeun Kwon,⁵ Jae-Young Choi,^{2,3†} Ho Bum Park^{1†}

Graphene is a distinct two-dimensional material that offers a wide range of opportunities for membrane applications because of ultimate thinness, flexibility, chemical stability, and mechanical strength. We demonstrate that few- and several-layered graphene and graphene oxide (GO) sheets can be engineered to exhibit the desired gas separation characteristics. Selective gas diffusion can be achieved by controlling gas flow channels and pores via different stacking methods. For layered (3- to 10-nanometer) GO membranes, tunable gas transport behavior was strongly dependent on the degree of interlocking within the GO stacking structure. High carbon dioxide/nitrogen selectivity was achieved by well-interlocked GO membranes in high relative humidity, which is most suitable for postcombustion carbon dioxide capture processes, including a humidified feed stream.

Compared with traditional systems, membrane-based gas separations have allowed much simpler, more efficient separations (1). One of the challenges of developing membrane materials is to achieve high flux as well as high selectivity. Because a membrane flux is inversely proportional to the thickness of selective layer (from tens of nanometers to several micrometers), a graphene sheet with one-atom thickness has been considered as the ultimate membrane platform. In its defect-free form, however, graphene exhibits perfect barrier properties to all molecules and ions (2, 3). Synthesis of large-area monolayer graphene by chemical vapor deposition (CVD) has recently been reported (4), and the resultant high-quality and uniform graphene sheet can be transferred to various substrates. We hypothesized that deposition of defect-free graphene sheets on gas-permeable polymer films would allow for preparation of flexible plastic barrier films to prevent gas and water vapor penetration. Therefore, we prepared polymer-supported thin films of graphene with varying numbers of deposited graphene sheets (Fig. 1A). Poly(1-methylsilyl-1-propyne) (PTMSP) was selected as the substrate because it is one of the most permeable, rigid, and glassy polymers available (5). The gas permeability of the resultant barrier films decreased as the number of

graphene layers deposited increased. However, a single graphene-layered PTMSP film (G1/PTMSP) showed a relatively small reduction in gas permeability compared with the original PTMSP (Fig. 1B), implying the presence of defects generated during the CVD growth and transfer process (Fig. 1C). These defects also resulted in a large electrical sheet resistance (>2500 ohms per square) (Fig. 1B), similar to values reported in the literature (6). This sheet resistance was considerably reduced (<1000 ohms per square) as more graphene layers were stacked. The reduction in sheet resistance correlated well with the gas permeability of the film (Fig. 1B).

We anticipated that the porosity caused by the graphene sheet defects could be mitigated by depositing more graphene layers. Although the gas permeability of the films decreased gradually with increasing number of graphene layers, we were not able to obtain excellent barrier films. Surprisingly, the O₂/N₂ selectivity of G#/PTMSP films (where # represents the number of graphene layers) improved (Fig. 1D) with increasing graphene stacking, which suggests that gases diffused not only through defective pores on the graphene sheet but also between the graphene interlayers (7–9), leading to higher selectivity than the original PTMSP film. The O₂/N₂ selectivity increased from 1.5 (for PTMSP) to 6 (for G5/PTMSP), whereas the O₂ permeability decreased from 730 to 29 barrer [1 barrer = 1×10^{-10} cm³ cm/cm²·sec·cmHg at standard temperature and pressure (STP)], surpassing the O₂/N₂ separation limitation of polymeric membranes (10) with a selectivity similar to that of carbon molecular sieve (CMS) membranes (11).

A possible explanation for this phenomenon is that the defective graphene sheets were irregularly aligned, resulting in the formation of gas-permeable, slit-like interlayer spacings. Pristine

graphene synthesized via CVD has a polycrystalline structure with grain boundaries linked to pentagon-heptagon pairs (12). Mechanical cleavage or oxidation at these sites can lead to sheet defects, such as tears or holes (12, 13), which allow ingress of gas molecules. Furthermore, because of the irregular stacking caused by corrugations, wrinkles, and ripples, randomly stacked graphene sheets had an interplanar spacing larger than that of closely packed graphitic layers (0.335 nm). The average interlayer spacing of randomly stacked graphene layers on a silicon wafer was 0.355 nm, albeit with a broad spacing distribution, as measured by two-dimensional (2D) grazing-incidence x-ray diffraction (fig. S1). The interlayer spacing of the graphene layers on PTMSP would be larger because the surface of this polymer film is not as flat as that of mica or silicon wafers (14). The increase in selectivity of the G#/PTMSP films, with the number of graphene layers deposited, indicates the formation of slit-like interlayer spacings of similar size to those present in CMS membranes.

We investigated the gas permeation behavior of thin graphene oxide (GO) membranes. GO's interlayer spacing is larger than that for graphene—namely, 0.6 to 1.0 nm—depending on the preparation method and the presence of water intercalated in the GO layers (15, 16). Thick (>10-nm) GO paper and thin (3- to 7-nm) GO membranes can be easily prepared by various solution methods, such as vacuum filtration, direct evaporation, and spray and spin coatings (16).

GO films in the dry state are not permeable to small gases (e.g., He), but water molecules permeate through hydrated GO films faster than expected (17). The potential energy barrier for gas entrance, tortuosity determined by the GO sheet size and thickness, layer structure, residual water content, and deformation of thin-layered structures during dewetting can all affect molecular transport in GO films. In contrast to graphene sheets, GO is not completely 2D (18), which suggests that GO should stack nonuniformly. For the above reasons, thin GO membranes should be permeable to small gases if sufficient transmembrane pressure is applied to overcome the energy barriers to pore entry and diffusion within pores or channels. In fact, we found that gas can permeate through even thick GO membranes at elevated transmembrane pressures and that the gas permeability can be tuned by changing the average GO sheet size (fig. S2). Furthermore, detectable gas permeance through the ultrathin GO membranes is strongly dependent on applied transmembrane pressure for a given average GO sheet size (fig. S3).

We used two different coating methods to prepare GO membranes on microporous polymeric membranes. The microporous polymeric support was selected to optimize both surface roughness and wettability to achieve a homogeneous coating. Among the commercially available supports, polyethersulfone (PES) membranes

¹Department of Energy Engineering, Hanyang University, Seoul 133-791, Republic of Korea. ²Samsung Advanced Institute of Technology (SAIT), Samsung Electronics, Suwon 446-712, Republic of Korea. ³Graphene Research Center at SAIT, Suwon 446-712, Republic of Korea. ⁴Department of Advanced Fiber Engineering, Inha University, Incheon 402-751, Republic of Korea. ⁵Samsung Corning Precision Materials, Ansan 336-725, Republic of Korea.

*These authors contributed equally to this work.

†Corresponding author. E-mail: jaeyoung88.choi@samsung.com (J.-Y.C.); badtzhb@hanyang.ac.kr (H.B.P.)

yielded the most uniform coating (fig. S4). We prepared several-layer GO membranes by contacting the support membrane surface to the air-liquid interface of a GO solution, followed by spin-coating (method one). We also prepared thin GO membranes by spin-casting of a GO solution on the membrane surface (method two) (fig. S5). Macroscopically, after several coatings, both membrane surfaces appeared to be homogeneously coated by a GO thin film without detectable pinholes or cracks under an optical microscope. The color of the GO-coated membranes (light yellow) differed from that of the pristine PES membrane surface (white) (fig. S6). Transmission electron microscopy (TEM) revealed that the coated GO thicknesses were ~3 to 7 nm (Fig. 2, A and B), depending on the number of coating iterations. We selected these GO coating methods to test the relative importance of electrostatic repulsion and immersion capillary force on the GO stacking structure. When considering GO deposition by method one, initial GO solution-membrane contact is performed without spin casting so that GO deposition is governed by electrostatic and hydrophilic interactions between the GO sheets and the polymer membrane. Generally, in an alkaline aqueous solution, GO sheet edges are negatively charged due to pendant ionized carboxylic acid groups. Therefore, GO sheet edge-to-edge interactions are repulsive, leading to an island-like assembly of GO sheets on the membrane surface. After this initial GO deposition on the membrane surface, water between deposited GO sheets and GO sheets in solution is removed through spin casting, allowing strong attractive capillary forces to further deposit GO sheets from solution. A second iteration of the dipping procedure in method one leads to further GO deposition through face-to-face attractive interactions (for instance, van der Waals interactions and hydrogen bonding) between the size-mismatched GO sheets (19). We found that this method leads to a relatively heterogeneous GO deposition (Fig. 2, C and E), in which the GO stacking structure resembles islands. In contrast, method two, in which the GO solution-membrane contact occurs only during spin casting, leads to a considerably more dense GO deposition. Presumably, this dense stacking occurs because the face-to-face attractive capillary forces created by the spin coating overcome the repulsive edge-to-edge GO sheet interaction. Therefore, the initial deposition is governed more by capillary interactions between the GO sheet faces and not the electrostatic interaction between the GO edges (Fig. 2, D and F). Cross-sectional TEM images show different stacking structures using each coating method (Fig. 2, A and B).

Gas permeance [in gas permeation units (GPUs), $1 \text{ GPU} = 1 \times 10^{-6} \text{ cm}^3/\text{cm}^2\cdot\text{sec}\cdot\text{cmHg}$ at STP] was measured at a feed pressure of 1 bar, using the common constant-pressure, variable-volume method. We found that the gas transport behavior of membranes prepared by methods one and two was very different. The GO membranes prepared by method one showed typical gas permeation

behavior explained by Knudsen transport of gases in nanoporous membranes (Fig. 3A). Here, gas transport is determined by the pore size of the membrane and the free path length of the molecules in a gas mixture, and Knudsen diffusion leads to separation of gases with large differences in their molecular weights (20). As expected, gas permeance decreased according to the molecular weight of the gases. The selectivity of membranes prepared by method one to He was in good agreement with theoretical Knudsen selectivity, but this did not hold true for their selectivity to CO_2 (Fig. 3A). During the permeation test at a feed pressure of 1 bar, CO_2 permeance decreased sharply with time and stabilized after 1 hour, whereas other gases did not show this reduction (Fig. 3B). Consequently, the small gas/ CO_2 selectivity of the membranes exceeded the Knudsen selectivity to a large extent; for example, the H_2/CO_2 selectivity was as high as 30, whereas the theoretical Knudsen selectivity of H_2/CO_2 is 4.67.

We now consider the role of both the porosity and chemical nature of the gas transport channels within the stacked GO structure. Highly interlocked, close-packed layering structures were not obtained, even after repeated coatings (fig. S7); rather, nanopores created by the edges of

noninterlocked GO sheets formed within the GO structure. Gas permeation occurred through these nanopores, as confirmed by the Knudsen transport behavior of the GO membranes. Furthermore, a number of carboxylic acid groups line the pore outer walls, because free carboxylic acid groups are distributed at GO edges. Torrisi *et al.* calculated the isotheric heats of sorption for CO_2 in functionalized metal-organic frameworks and predicted that the incorporation of a carboxylic acid group would lead to the highest isotheric heat (21). Although CO_2 is a nonpolar gas, the polarity of the individual C-O bonds in the molecule allows for interaction with polar groups in GO. Thus, CO_2 can act as a Lewis acid or a Lewis base and can participate in hydrogen bonding. Carboxylic acid groups provide a preferential site for CO_2 adsorption, consequently retarding CO_2 transport in the nanopores by strongly trapping CO_2 molecules. This phenomenon is counterintuitive, because strong affinity between penetrant and nanopore walls often leads to surface diffusion of the penetrant preferentially sorbed on the pore wall, resulting in flux enhancement of highly sorbed or condensable penetrants (22).

Because GO is hydrophilic, we also evaluated the gas transport behavior of GO membranes

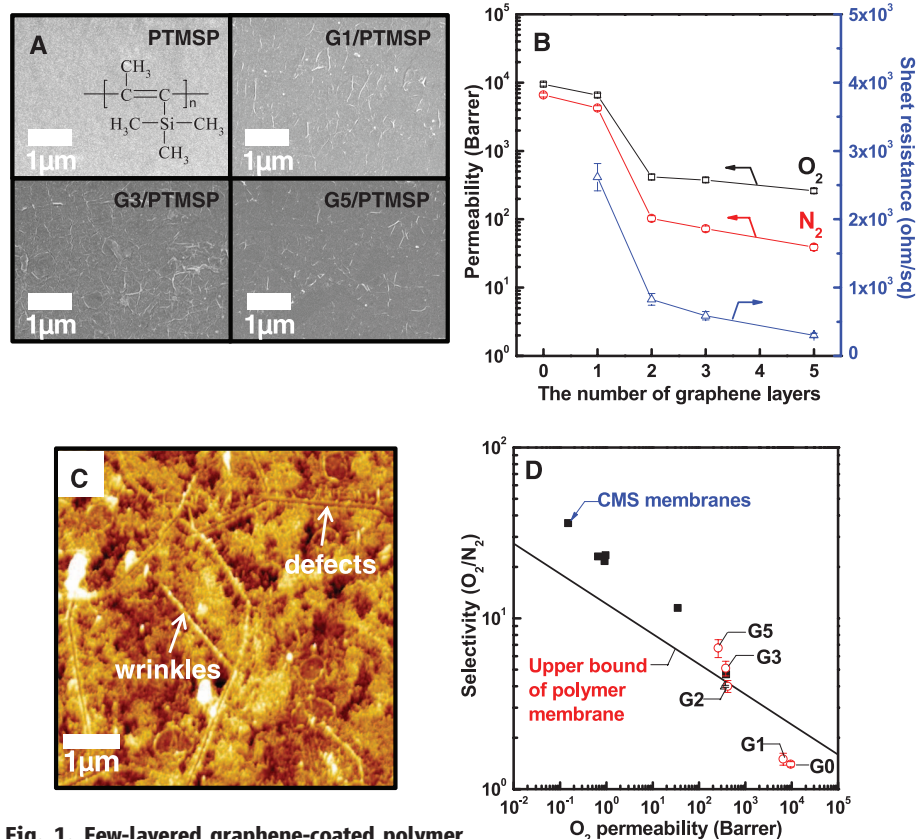


Fig. 1. Few-layered graphene-coated polymer membranes. (A) Scanning electron microscopy (SEM)

images of graphene/PTMSP membrane surfaces. (B) Changes in gas permeability and sheet resistance of graphene/PTMSP membranes as a function of the number of graphene layers. Error bars indicate the SD of all raw data. (C) Atomic force microscopy (AFM) images of a graphene/PTMSP membrane surface. (D) Relation between O_2 permeability and O_2/N_2 selectivity of graphene/PTMSP membranes. The upper bound is from (10), as well as gas separation performance data of CMS membranes reported in the literature (11). Error bars indicate the SD in the current measured from five parallel experiments.

Fig. 2. Ultrathin GO membranes.

Cross-sectional TEM images of ultrathin GO membranes. (A) Method one; (B) method two. SEM images of GO films coated on an SiO_x wafer (here, x indicates the number of O atoms). (C) Method one; (D) method two. AFM images of GO membrane surfaces and inset figures showing depth profiles of GO membrane surfaces. (E) Method one [root mean square roughness (R_q) = 0.8 nm, average roughness (R_a) = 0.614 nm]; (F) method two (R_q = 0.608 nm, R_a = 0.467 nm).

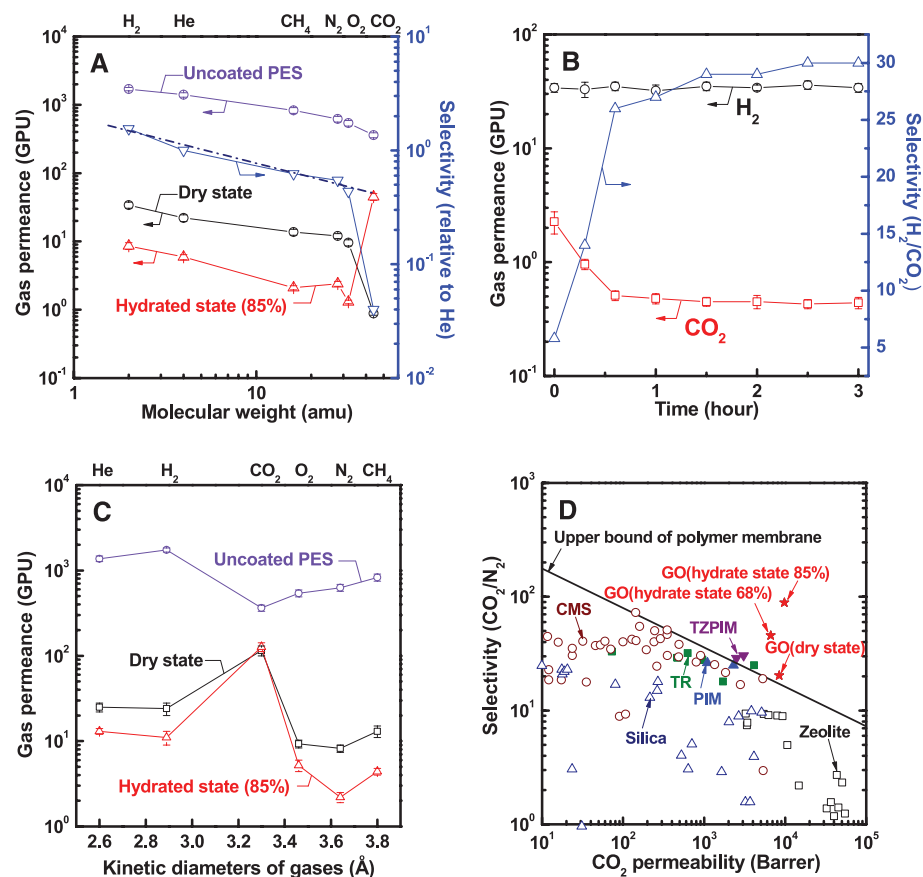
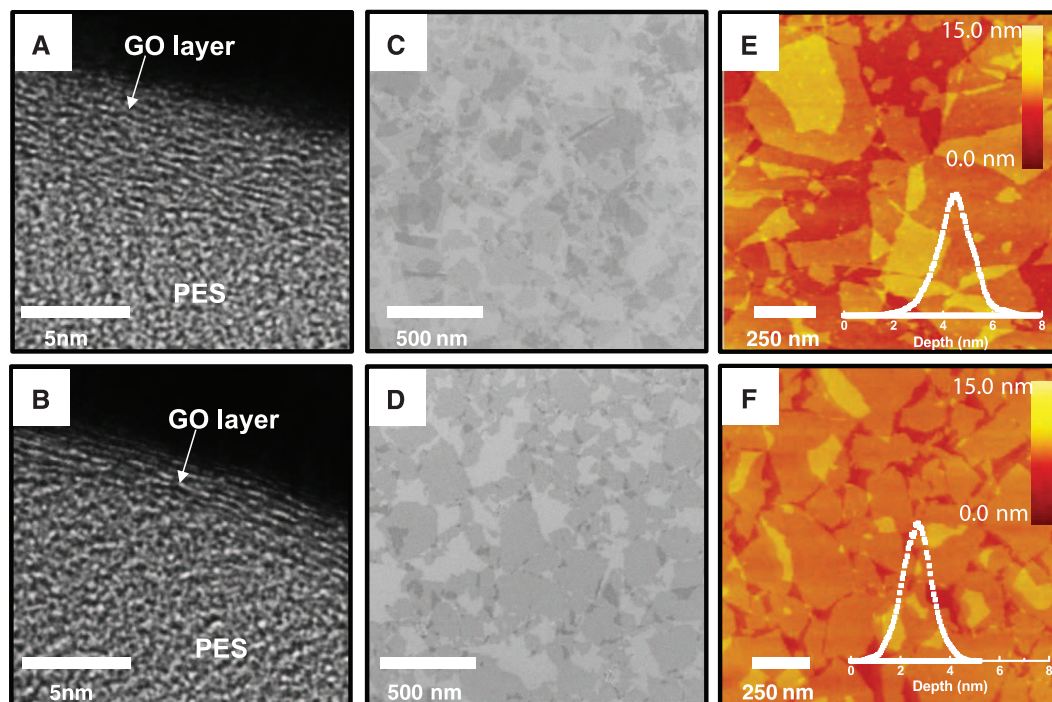


Fig. 3. Gas transport behavior through ultrathin GO membranes. (A) Gas permeances of GO membranes as a function of molecular weight (method one; dashed line represents the ideal Knudsen selectivity) under dry and humidified conditions. amu, atomic mass unit. (B) H_2 and CO_2 permeances and H_2/CO_2 selectivity of method one GO membranes as a function of permeation time. (C) Gas permeances of GO membranes as a function of kinetic diameter (method two) under dry and humidified conditions. (D) Relation between CO_2 permeability and CO_2/N_2 selectivity of method two GO membranes under dry and humidified conditions [TR (23); tetrazole PIM (TZPIM) and PIM (25); CMS (26)]. Error bars indicate the SD of all raw data.

(method one) by changing the relative humidity of the feed streams (Fig. 3A). In general, all gas permeances decreased as the humidity in the feed increased, implying that condensed water molecules in the pores or between layers hindered the transport of noncondensable small gas molecules. In contrast, CO_2 permeance was 50 times higher in the presence of water, leading to an enhancement in the selectivities of gas pairs of interest (for example, CO_2/CH_4 , CO_2/H_2 , and CO_2/N_2) (fig. S8).

GO membranes prepared by method two formed highly interlocked layer structures and also exhibited extraordinary gas permeation behavior, albeit different than the behavior exhibited by membranes prepared via method one. For these GO membranes, the gas permeance order at 298 K was $\text{CO}_2 > \text{H}_2 \geq \text{He} > \text{CH}_4 > \text{O}_2 > \text{N}_2$ (Fig. 3C). This order has been observed for a few high free-volume microporous glassy polymers, such as PTMSP, that consist of a loosely packed network of polymer chains with continuous channels or pores for diffusion (5, 23). The gas transport behavior of these GO membranes means that they comprised closed-packed, interlocked GO layered structures. These GO membranes were less gas permeable than those prepared by method one but were also more selective, indicating that gases diffused selectively between the GO interlayers and, thus, had a different effective diffusion pathway. Based on the resistance model (24), we calculated the intrinsic gas permeability of the thin GO membranes. The CO_2 permeability is ~ 8500 barrer, and CO_2/N_2 selectivity is ~ 20 . The separation performance is higher than that reported for CO_2 for polymeric membranes, including thermally rearranged (TR) polymers (23), polymers of intrinsic microporosity

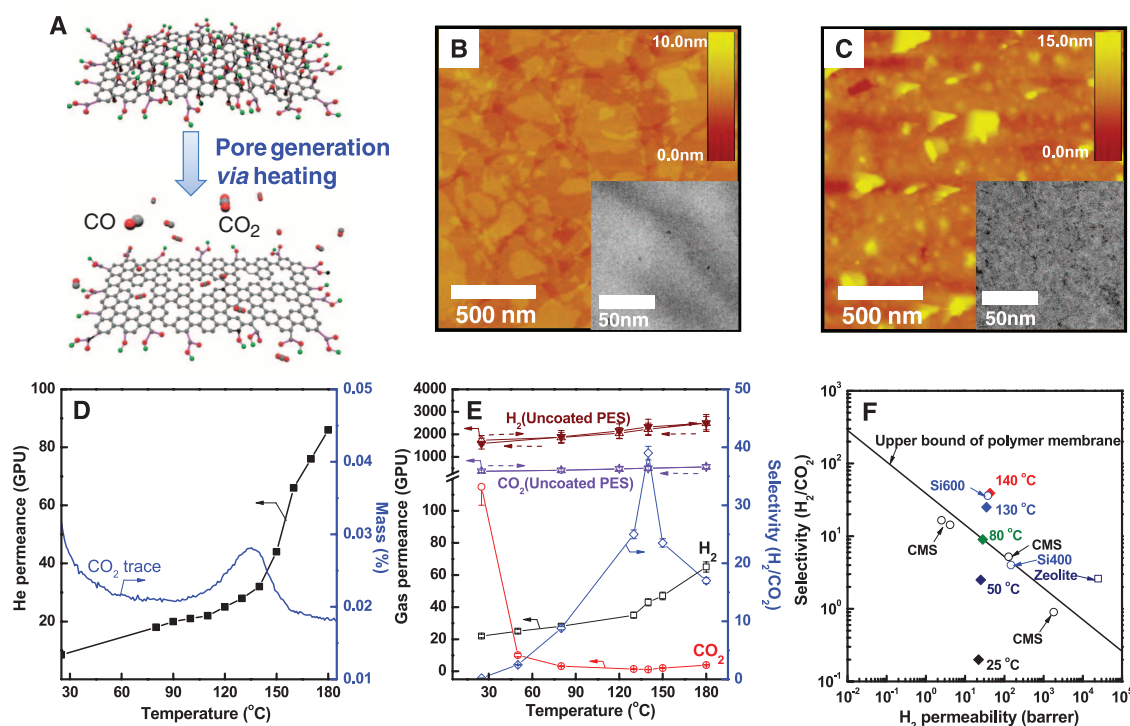


Fig. 4. Gas transport behavior through thermally reduced GO membranes. (A) Schematic illustration of pore generation in GO membranes using a thermal treatment process. AFM images of method two GO membranes (B) before ($R_q = 0.553$ nm, $R_a = 0.450$ nm) and (C) after thermal treatment at 180°C for 3 hours ($R_q = 1.75$ nm, $R_a = 1.09$ nm). The insets to (B) and (C), respectively, show TEM images before and after thermal treatment at 180°C

for 3 hours. (D) He permeance trend and CO₂ trace of mass spectroscopy as a function of temperature. (E) H₂ and CO₂ permeability of thermally treated GO membranes as a function of temperature. Error bars indicate the SD of all raw data. (F) Comparison of gas separation performance between GO membranes and other membranes in the literature [polymers (10); CMS (26, 29); zeolite (26); silica (30)].

(PIM) (25), and inorganic membranes, such as carbon, silica, and zeolite (26) (Fig. 3D). The gas permeances decreased gradually as the humidity increased (27), but the CO₂ permeance remained constant, irrespective of the relative humidity (fig. S9), resulting in highly CO₂-selective, ultrathin (<10-nm) carbon membranes (Fig. 3D and fig. S10).

More permeable, selective graphene-based membranes can be prepared by creating pores on the basal plane by simple heat treatment (Fig. 4, A to C). Thermal treatment of ultrathin GO membranes (prepared with method two) caused decomposition accompanied by the release of, primarily, water and CO₂ at a temperature of ~140°C, as confirmed by analysis with a thermogravimetric analyzer–mass spectrometer (Fig. 4D), consistent with the literature (28). At this temperature, CO and CO₂ are released from oxygen-containing functional groups on basal planes or at edges, resulting in irreversible defects. After the membrane was mounted in a cell surrounded by a heating chamber, the gas permeation rates of H₂ and CO₂ at a feed pressure of 1 bar were recorded as a function of cell temperature. Below 130°C, H₂ permeance increased gradually, whereas CO₂ permeance decreased steadily. At ~130° to 140°C, H₂ permeance increased abruptly and CO₂ permeance increased slightly (Fig. 4E), implying that thermal annealing made the microstructures of the GO active layers more porous due to irre-

versible pore formation. Without such structural deformation, the slope of gas permeance versus temperature should be linearly positive (for H₂) or negative (for CO₂) under thermally activated diffusion conditions. However, two distinct slopes were observed, presenting evidence of a more open porous structure. The gradual reduction in CO₂ permeance below 130°C is due to the low enthalpy of sorption. The increase in CO₂ permeance above 140°C also indicates more porous microstructures in the GO thin layers. The H₂/CO₂ permselectivity at 140°C was as high as 40 (Fig. 4F), which is one of the highest values reported for other comparable membranes (10, 26, 29, 30).

Overall, transport in graphene or GO nanosheets has been covered less than that in carbon nanotubes, but there is great potential and the research area is growing. The ability to have adaptive and dynamic layer distances is a distinct attribute of stacked graphene or GO nanosheets. With careful control of the stacking structures, separation factors and flow rates can be further improved. Indeed, several layered GO membranes behave as either nanoporous (method one) or molecular-sieving membranes (method two) by the stacking method. Both membranes show CO₂-philic permeation behavior, which is further enhanced by the presence of water. As such, these membranes are promising materials for industrial CO₂ separation processes related to petrochemical engineering (CO₂ removal from natural gas), the

environment (CO₂ capture from flue gas), and biomass energy (CO₂ recovery from landfill gas).

References and Notes

1. H. Strathmann, *Introduction to Membrane Science and Technology* (Wiley, Weinheim, Germany, 2011).
2. J. S. Bunch *et al.*, *Nano Lett.* **8**, 2458–2462 (2008).
3. O. Leenaerts, B. Partoens, F. M. Peeters, *Appl. Phys. Lett.* **93**, 193107 (2008).
4. X. Li *et al.*, *Science* **324**, 1312–1314 (2009).
5. T. Masuda, E. Isobe, T. Higashimura, K. Takada, *J. Am. Chem. Soc.* **105**, 7473–7474 (1983).
6. X. S. Li *et al.*, *Nano Lett.* **9**, 4359–4363 (2009).
7. D. E. Jiang, V. R. Cooper, S. Dai, *Nano Lett.* **9**, 4019–4024 (2009).
8. S. Blankenburg *et al.*, *Small* **6**, 2266–2271 (2010).
9. J. Schrier, *Am. Chem. Soc. Appl. Mater. Interfaces* **4**, 3745–3752 (2012).
10. L. M. Robeson, *J. Membr. Sci.* **320**, 390–400 (2008).
11. H. Suda, K. Haraya, *J. Phys. Chem. B* **101**, 3988–3994 (1997).
12. P. Y. Huang *et al.*, *Nature* **469**, 389–392 (2011).
13. D. L. Duong *et al.*, *Nature* **490**, 235–239 (2012).
14. C. H. Lui, L. Liu, K. F. Mak, G. W. Flynn, T. F. Heinz, *Nature* **462**, 339–341 (2009).
15. D. A. Dikin *et al.*, *Nature* **448**, 457–460 (2007).
16. Y. W. Zhu *et al.*, *Adv. Mater.* **22**, 3906–3924 (2010).
17. R. R. Nair, H. A. Wu, P. N. Jayaram, I. V. Grigorieva, A. K. Geim, *Science* **335**, 442–444 (2012).
18. M. S. Spector, E. Naranjo, S. Chiruvolu, J. A. Zasadzinski, *Phys. Rev. Lett.* **73**, 2867–2870 (1994).
19. S. K. Bhatia, M. R. Bonilla, D. Nicholson, *Phys. Chem. Chem. Phys.* **13**, 15350–15383 (2011).

20. L. J. Cote, F. Kim, J. X. Huang, *J. Am. Chem. Soc.* **131**, 1043–1049 (2009).
21. A. Torrisi, C. Mellot-Draznieks, R. G. Bell, *J. Chem. Phys.* **132**, 044705 (2010).
22. M. B. Rao, S. Sircar, *J. Membr. Sci.* **85**, 253–264 (1993).
23. H. B. Park *et al.*, *Science* **318**, 254–258 (2007).
24. I. Pinnau, W. J. Koros, *Ind. Eng. Chem. Res.* **30**, 1837–1840 (1991).
25. N. Y. Du *et al.*, *Nat. Mater.* **10**, 372–375 (2011).
26. D. Shekhawat, D. R. Luebke, H. W. Pennline, “A review of carbon dioxide selective membranes: A topical report” (Report DOE/NETL 2003/1200, U.S. Department of Energy, National Energy Technology Laboratory, Pittsburgh, PA, 2003).
27. J. C. Poshusta, R. D. Noble, J. L. Falconer, *J. Membr. Sci.* **186**, 25–40 (2001).
28. S. Eigler, C. Dotzer, A. Hirsch, M. Enzelberger, P. Muller, *Chem. Mater.* **24**, 1276–1282 (2012).
29. M. B. Shiflett, H. C. Foley, *Science* **285**, 1902–1905 (1999).
30. R. M. de Vos, H. Verweij, *Science* **279**, 1710–1711 (1998).

Acknowledgments: This work was mainly supported by Korea Carbon Capture and Sequestration Research and Development Center (KCRC) (grant no. 2012-0008907) funded by the Korea government (Ministry of Science, Information Communication Technology and Future Planning). We thank the National Synchrotron Light Source

at Brookhaven National Laboratory for providing the X9 beam line (grant no. DE-AC02-98CH10886) and B. D. McCloskey for thoughtful reading and commentary on our paper. H.B.P. also acknowledges support by the research fund of Hanyang University (grant no. 200900000001052).

Supplementary Materials

www.sciencemag.org/content/342/6154/91/suppl/DC1
Materials and Methods

Figs. S1 to S10
References (31, 32)

4 February 2013; accepted 4 September 2013
10.1126/science.1236098

Ultrathin, Molecular-Sieving Graphene Oxide Membranes for Selective Hydrogen Separation

Hang Li,^{1,2} Zhuonan Song,^{1,2} Xiaojie Zhang,^{1,2} Yi Huang,^{1,2} Shiguang Li,³ Yating Mao,¹ Harry J. Ploehn,¹ Yu Bao,⁴ Miao Yu^{1,2*}

Ultrathin, molecular-sieving membranes have great potential to realize high-flux, high-selectivity mixture separation at low energy cost. Current microporous membranes [pore size < 1 nanometer (nm)], however, are usually relatively thick. With the use of current membrane materials and techniques, it is difficult to prepare microporous membranes thinner than 20 nm without introducing extra defects. Here, we report ultrathin graphene oxide (GO) membranes, with thickness approaching 1.8 nm, prepared by a facile filtration process. These membranes showed mixture separation selectivities as high as 3400 and 900 for H₂/CO₂ and H₂/N₂ mixtures, respectively, through selective structural defects on GO.

Zeolites (1, 2), silica (3), carbon (4), and polymers (5) have been made into microporous membranes that have shown promising gas mixture separation performance. These membranes separate mixtures on the basis of selective adsorption, diffusion rate differences, or molecular-sieving mechanisms. Current microporous membranes, however, are usually thicker than 20 nm to minimize undesirable flux contribution through non-selective membrane defects, and they maintain reasonably high separation selectivity.

Graphene-based materials, such as graphene and graphene oxide (GO), have been considered promising membrane materials, because they are only one carbon atom thick and, thus, may form separation membranes that minimize transport resistance and maximize flux. Additionally, they have good stability (6, 7) and are mechanically strong (8). Graphene-based materials have been made into centimeter-sized, thick (~1-μm) membranes and micrometer-sized, isolated single sheets for pure component permeation studies where

they were found to be either impermeable to small gas molecules or not practical for separation applications (9–12).

We used single-layered GO flakes, prepared by the modified Hummer's method (13). Ultrathin GO membranes were prepared by vacuum filtration, as described in detail in fig. S1. Centrifugation and dilution of GO dispersions were found to be important for preparing high-quality GO membranes [fig. S2 and discussion in (13)]. Figure 1A shows a ~9-nm-thick GO membrane with a permeation area of ~4 cm² on anodic aluminum oxide (AAO) support. A glass membrane module was used for gas permeation-separation experiments, as shown schematically in fig. S3. X-ray diffraction shows the characteristic peak of GO at 2θ of 11.1° [fig. S4 and analysis in (13)], and GO flakes are ~500 nm in size and single-layered, as confirmed by atomic force microscopy (AFM) (Fig. 1B), which also shows the height profile of a GO flake (Fig. 1C). In Fig. 1, panels D and E show the surface of an 18-nm-thick GO membrane on AAO. Compared with the AAO support (Fig. 1F), a very thin GO coating can be seen. We deposited a relatively thick GO membrane (~180 nm (Fig. 1G)) to correlate the GO deposition with the membrane thickness. GO dispersion for this 180-nm-membrane preparation was then diluted 100, 20, and 10 times to obtain the above 1.8-, 9-, and 18-nm-thick GO

membranes, respectively, assuming no GO loss during filtration and constant membrane density. We used x-ray photoelectron spectroscopy (XPS) to detect surface elements for these ultrathin GO membranes on AAO (Fig. 1, H and I). For a 1.8-nm-thick membrane, a substantial amount of aluminum can be seen, because the mean free path of excited electrons is longer than the surface GO membrane thickness. However, for thicker membranes (9 and 18 nm), much smaller amounts of the underlying aluminum can be seen, because GO thickness is larger than the excited electron mean free path. This finding is consistent with surface carbon detection by XPS as well (Fig. 1I). See the supplementary materials for a detailed analysis.

We conducted permeation tests with different light gas molecules to probe pore sizes. Hydrogen (kinetic diameter: 0.289 nm) permeated ~300 times faster than did CO₂ (0.33 nm) through a ~18-nm-thick GO membrane at 20°C (Fig. 2A). Their kinetic diameter difference is only 0.04 nm, suggesting that the average size of pores for permeation in the GO membrane may be between 0.289 and 0.33 nm. O₂ and N₂ showed similar permeance as CO₂. However, CO and CH₄ had slightly higher permeance, although these molecules are slightly larger than the aforementioned ones. Koenig *et al.* (12) also found that CH₄ had slightly higher permeance than N₂ through pristine graphene flakes, though the reason is still unclear. Figure 2B shows H₂ and He permeances for GO membranes with different thicknesses. Gas permeance is usually inversely proportional to the membrane thickness for conventional membranes (14). Surprisingly, we found that H₂ and He permeances decrease exponentially as membrane thickness increases from 1.8 to 180 nm (Fig. 2B). We speculate that the major transport pathway for these molecules is selective structural defects within GO flakes, instead of spacing between GO flakes. Reduction has been shown as an effective way to narrow interlayer spacing in GO membranes and, thus, limit permeation of molecules through spacing (10). We reduced GO membranes with thickness from 1.8 to 20 nm and conducted pressure-driven water permeation. We found that water permeance decreased approximately three orders of magnitude: For example, water permeance through a 3-nm GO membranes was 1370 liters/(m²·hour·bar), whereas it was 0.5 to 1 liters/(m²·hour·bar) through

¹Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA. ²Catalysis for Renewable Fuels Center, University of South Carolina, Columbia, SC 29208, USA. ³Gas Technology Institute, 1700 South Mount Prospect Road, Des Plaines, IL 60018, USA. ⁴College of Applied Science and Technology, Rochester Institute of Technology, Rochester, NY 14623, USA.

*Corresponding author. E-mail: yumiao@cec.sc.edu



Ultrathin, Molecular-Sieving Graphene Oxide Membranes for Selective Hydrogen Separation

Hang Li *et al.*

Science **342**, 95 (2013);

DOI: 10.1126/science.1236686

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/95.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.95.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/95.full.html#related>

This article **cites 39 articles**, 6 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/95.full.html#ref-list-1>

20. L. J. Cote, F. Kim, J. X. Huang, *J. Am. Chem. Soc.* **131**, 1043–1049 (2009).
21. A. Torrisi, C. Mellot-Draznieks, R. G. Bell, *J. Chem. Phys.* **132**, 044705 (2010).
22. M. B. Rao, S. Sircar, *J. Membr. Sci.* **85**, 253–264 (1993).
23. H. B. Park *et al.*, *Science* **318**, 254–258 (2007).
24. I. Pinnau, W. J. Koros, *Ind. Eng. Chem. Res.* **30**, 1837–1840 (1991).
25. N. Y. Du *et al.*, *Nat. Mater.* **10**, 372–375 (2011).
26. D. Shekhawat, D. R. Luebke, H. W. Pennline, “A review of carbon dioxide selective membranes: A topical report” (Report DOE/NETL 2003/1200, U.S. Department of Energy, National Energy Technology Laboratory, Pittsburgh, PA, 2003).
27. J. C. Poshusta, R. D. Noble, J. L. Falconer, *J. Membr. Sci.* **186**, 25–40 (2001).
28. S. Eigler, C. Dotzer, A. Hirsch, M. Enzelberger, P. Muller, *Chem. Mater.* **24**, 1276–1282 (2012).
29. M. B. Shiflett, H. C. Foley, *Science* **285**, 1902–1905 (1999).
30. R. M. de Vos, H. Verweij, *Science* **279**, 1710–1711 (1998).

Acknowledgments: This work was mainly supported by Korea Carbon Capture and Sequestration Research and Development Center (KCRC) (grant no. 2012-0008907) funded by the Korea government (Ministry of Science, Information Communication Technology and Future Planning). We thank the National Synchrotron Light Source

at Brookhaven National Laboratory for providing the X9 beam line (grant no. DE-AC02-98CH10886) and B. D. McCloskey for thoughtful reading and commentary on our paper. H.B.P. also acknowledges support by the research fund of Hanyang University (grant no. 200900000001052).

Supplementary Materials

www.sciencemag.org/content/342/6154/91/suppl/DC1
Materials and Methods

Figs. S1 to S10
References (31, 32)

4 February 2013; accepted 4 September 2013
10.1126/science.1236098

Ultrathin, Molecular-Sieving Graphene Oxide Membranes for Selective Hydrogen Separation

Hang Li,^{1,2} Zhuonan Song,^{1,2} Xiaojie Zhang,^{1,2} Yi Huang,^{1,2} Shiguang Li,³ Yating Mao,¹ Harry J. Ploehn,¹ Yu Bao,⁴ Miao Yu^{1,2*}

Ultrathin, molecular-sieving membranes have great potential to realize high-flux, high-selectivity mixture separation at low energy cost. Current microporous membranes [pore size < 1 nanometer (nm)], however, are usually relatively thick. With the use of current membrane materials and techniques, it is difficult to prepare microporous membranes thinner than 20 nm without introducing extra defects. Here, we report ultrathin graphene oxide (GO) membranes, with thickness approaching 1.8 nm, prepared by a facile filtration process. These membranes showed mixture separation selectivities as high as 3400 and 900 for H₂/CO₂ and H₂/N₂ mixtures, respectively, through selective structural defects on GO.

Zeolites (1, 2), silica (3), carbon (4), and polymers (5) have been made into microporous membranes that have shown promising gas mixture separation performance. These membranes separate mixtures on the basis of selective adsorption, diffusion rate differences, or molecular-sieving mechanisms. Current microporous membranes, however, are usually thicker than 20 nm to minimize undesirable flux contribution through non-selective membrane defects, and they maintain reasonably high separation selectivity.

Graphene-based materials, such as graphene and graphene oxide (GO), have been considered promising membrane materials, because they are only one carbon atom thick and, thus, may form separation membranes that minimize transport resistance and maximize flux. Additionally, they have good stability (6, 7) and are mechanically strong (8). Graphene-based materials have been made into centimeter-sized, thick (~1-μm) membranes and micrometer-sized, isolated single sheets for pure component permeation studies where

they were found to be either impermeable to small gas molecules or not practical for separation applications (9–12).

We used single-layered GO flakes, prepared by the modified Hummer's method (13). Ultrathin GO membranes were prepared by vacuum filtration, as described in detail in fig. S1. Centrifugation and dilution of GO dispersions were found to be important for preparing high-quality GO membranes [fig. S2 and discussion in (13)]. Figure 1A shows a ~9-nm-thick GO membrane with a permeation area of ~4 cm² on anodic aluminum oxide (AAO) support. A glass membrane module was used for gas permeation-separation experiments, as shown schematically in fig. S3. X-ray diffraction shows the characteristic peak of GO at 2θ of 11.1° [fig. S4 and analysis in (13)], and GO flakes are ~500 nm in size and single-layered, as confirmed by atomic force microscopy (AFM) (Fig. 1B), which also shows the height profile of a GO flake (Fig. 1C). In Fig. 1, panels D and E show the surface of an 18-nm-thick GO membrane on AAO. Compared with the AAO support (Fig. 1F), a very thin GO coating can be seen. We deposited a relatively thick GO membrane (~180 nm (Fig. 1G)) to correlate the GO deposition with the membrane thickness. GO dispersion for this 180-nm-membrane preparation was then diluted 100, 20, and 10 times to obtain the above 1.8-, 9-, and 18-nm-thick GO

membranes, respectively, assuming no GO loss during filtration and constant membrane density. We used x-ray photoelectron spectroscopy (XPS) to detect surface elements for these ultrathin GO membranes on AAO (Fig. 1, H and I). For a 1.8-nm-thick membrane, a substantial amount of aluminum can be seen, because the mean free path of excited electrons is longer than the surface GO membrane thickness. However, for thicker membranes (9 and 18 nm), much smaller amounts of the underlying aluminum can be seen, because GO thickness is larger than the excited electron mean free path. This finding is consistent with surface carbon detection by XPS as well (Fig. 1I). See the supplementary materials for a detailed analysis.

We conducted permeation tests with different light gas molecules to probe pore sizes. Hydrogen (kinetic diameter: 0.289 nm) permeated ~300 times faster than did CO₂ (0.33 nm) through a ~18-nm-thick GO membrane at 20°C (Fig. 2A). Their kinetic diameter difference is only 0.04 nm, suggesting that the average size of pores for permeation in the GO membrane may be between 0.289 and 0.33 nm. O₂ and N₂ showed similar permeance as CO₂. However, CO and CH₄ had slightly higher permeance, although these molecules are slightly larger than the aforementioned ones. Koenig *et al.* (12) also found that CH₄ had slightly higher permeance than N₂ through pristine graphene flakes, though the reason is still unclear. Figure 2B shows H₂ and He permeances for GO membranes with different thicknesses. Gas permeance is usually inversely proportional to the membrane thickness for conventional membranes (14). Surprisingly, we found that H₂ and He permeances decrease exponentially as membrane thickness increases from 1.8 to 180 nm (Fig. 2B). We speculate that the major transport pathway for these molecules is selective structural defects within GO flakes, instead of spacing between GO flakes. Reduction has been shown as an effective way to narrow interlayer spacing in GO membranes and, thus, limit permeation of molecules through spacing (10). We reduced GO membranes with thickness from 1.8 to 20 nm and conducted pressure-driven water permeation. We found that water permeance decreased approximately three orders of magnitude: For example, water permeance through a 3-nm GO membranes was 1370 liters/(m²·hour·bar), whereas it was 0.5 to 1 liters/(m²·hour·bar) through

¹Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA. ²Catalysis for Renewable Fuels Center, University of South Carolina, Columbia, SC 29208, USA. ³Gas Technology Institute, 1700 South Mount Prospect Road, Des Plaines, IL 60018, USA. ⁴College of Applied Science and Technology, Rochester Institute of Technology, Rochester, NY 14623, USA.

*Corresponding author. E-mail: yumiao@cec.sc.edu

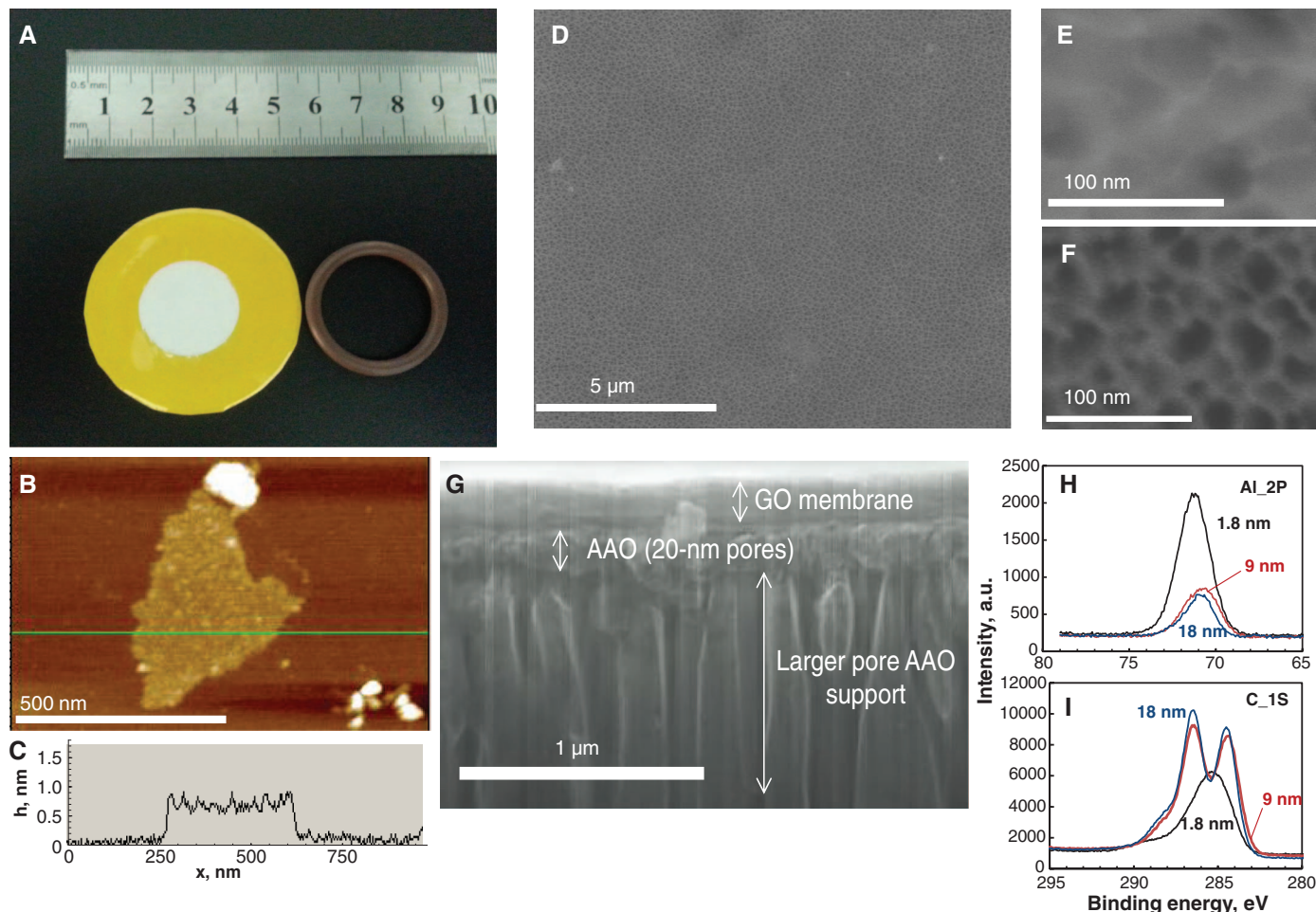


Fig. 1. GO membranes supported on porous AAO. (A) Digital picture of an ultrathin GO membrane on AAO (~9 nm). The white circular area is the permeation area (~4 cm²) with supported GO; the yellow Kapton tape is used for GO protection and sealing by an O-ring during permeation measurements. (B) AFM image of a GO flake on freshly cleaved mica. (C) The height profile across the green line in (B) is shown here. *h*, height; *x*, position. (D) Field-emission scanning electron microscopy (FE-SEM) image

of the surface of a GO membrane (~18 nm thick) on porous AAO. (E) FE-SEM image of the GO membrane surface (~18 nm thick) with higher magnification. (F) FE-SEM image of the AAO surface without the GO membrane. (G) FE-SEM image of the cross-sectional view of a thick GO membrane (~180 nm). (H) Al 2P and (I) C 1S XPS spectra of ultrathin GO membranes (~1.8, 9, and 18 nm thick) supported on porous AAO. a.u., arbitrary units.

a reduced GO membrane. This observation is in agreement with the findings of Nair *et al.* (10) and suggests that interlayer spacing has been eliminated or considerably narrowed by reduction. We then measured single-gas permeation through 18-nm reduced GO membranes (fig. S5) but found no obvious gas permeance change, which suggests that interlayer spacing is not the major transport pathway and permeation of molecules is attributed to the selective structural defects within GO flakes. Exponential dependence of gas permeances on membrane thickness (Fig. 2B) may result from the particular molecular transport pathway through the selective structural defects in layered GO membranes. Various defects on graphene have been found capable of separating H₂ from other small molecules (N₂, CH₄, etc.) (15–17). The molecular-sieving behavior of H₂ over other molecules may be attributed to the intrinsic defects in our membranes, as supported by the Raman spectrum [fig. S6 and analysis in (13)]. Koenig *et al.*

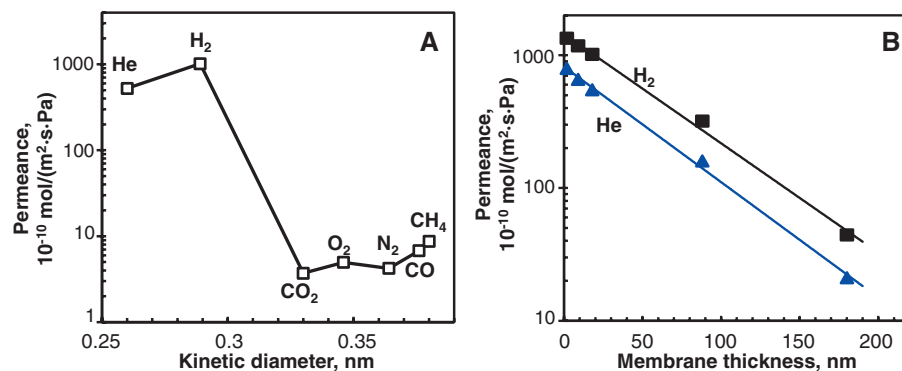


Fig. 2. Single-gas permeation through GO membranes supported on porous AAO at 20°C. (A) Permeances of seven molecules through a ~18-nm-thick GO membrane. (B) Permeances of H₂ and He through GO membranes with different thicknesses. The lines in (B) denote exponential fits.

(12) found that H₂/N₂ ideal selectivity for isolated graphene sheets was higher than 10,000 after etching graphene by ultraviolet-induced oxidation. We

noticed that, before etching, some of their graphene sheets showed high ideal selectivities for H₂/CH₄ (~100) and H₂/N₂ (~100), indicating that intrinsic

defects may also have decent molecular-sieving behavior. Our single-gas permeation results were consistent with their observation. We also used an exponential fit to extrapolate He permeance for a 1- μm -thick GO membrane (see Fig. 2B) and found that it is, appropriately, 10^{-16} mol/($\text{m}^2\cdot\text{s}\cdot\text{Pa}$). This

explains why the 1- μm -thick GO membranes prepared by Nair *et al.* were impermeable to He (10). Separation of H_2 from other small molecules has important applications, such as precombustion CO_2 capture and H_2 recovery for ammonia production (18–21).

Separation selectivity and permeance are two important parameters to evaluate membrane separation performance. We first conducted a control experiment for an AAO support. We found that the gas permeances were high [$>10^{-6}$ mol/($\text{m}^2\cdot\text{s}\cdot\text{Pa}$)] and selectivities of H_2 over CO_2 and N_2 were low (<5), as expected for Knudsen diffusion through 20-nm pores. Figure 3 shows separation results for 50:50 H_2/CO_2 and 50:50 H_2/N_2 mixtures for 1.8-, 9-, and 18-nm thick GO membranes. All the GO membranes showed high H_2/CO_2 selectivity (>2000) at 20°C , with a value of 3400 for the 9-nm-thick membrane. This is unusual, because microporous membranes reported in the literature showed low H_2/CO_2 selectivity (<10) or were selective to CO_2 over H_2 at temperatures below 100°C due to strong CO_2 adsorption and blocking of H_2 permeation (22–24). Adsorption isotherms on GO powder at 20°C showed much stronger CO_2 adsorption than H_2 adsorption (fig. S7). These results suggest a molecular-sieving separation of H_2 from CO_2 , because strongly adsorbed CO_2 on GO flakes has negligible effects on H_2 permeation, which means that CO_2 cannot fit into most of the GO structural defects that only allow H_2 permeation. CO_2 seems to permeate through a very small number of larger structural defects. The observed H_2/CO_2 separation selectivity was higher than the ideal selectivity, implying that the larger defects are also selective for H_2 over CO_2 , probably due to the smaller size of H_2 . H_2/CO_2 separation selectivity decreased with increasing temperature, resulting from the faster increase of CO_2 permeance than that of H_2 . But even at 100°C , H_2/CO_2 selectivity was still 250 for the 18-nm-thick membrane. This suggests a more activated CO_2 diffusion than that of H_2 through GO membranes, resulting from the tight fit of CO_2 molecules in these defects [fig. S8 and analysis in (13)]. H_2/N_2 mixture separation showed a similar behavior, and the highest selectivity is ~ 900 for the 9-nm GO membrane at 20°C . We have prepared at least three GO membranes for each thickness and obtained good reproducibility; variation of membrane permeation performance is within 15% for all membranes. We also fabricated ultrathin GO membranes on low-cost cellulose acetate supports (100-nm pores) and obtained similar separation performance. For example, for a ~ 18 -nm-thick GO membrane on cellulose acetate support, H_2/CO_2 and H_2/N_2 separation selectivities are 1110 and 300, respectively. Figure 3G shows a comparison of ultrathin GO membranes with polymeric membranes and inorganic membranes for H_2/CO_2 mixture separation. Typically, for separation using polymeric membranes, permeance decreases as separation selectivity increases. An upper bound can typically be used to compare the separation performance of a new membrane with that of previous membranes. Ultrathin GO membranes are far above the upper bound for polymeric membranes (black line) and show superior separation performance compared with representative

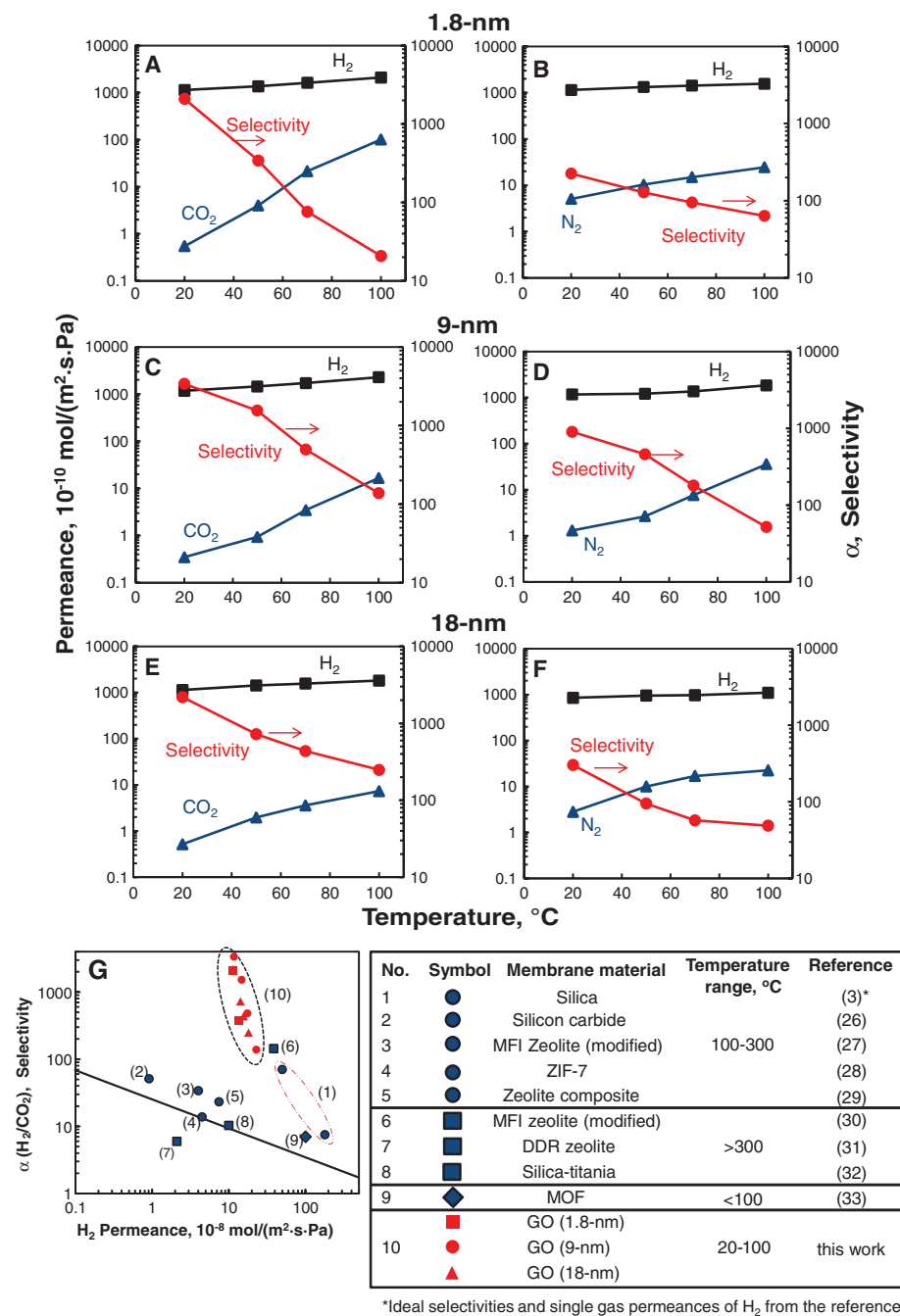


Fig. 3. 50:50 H_2/CO_2 and H_2/N_2 gas mixture separations and comparison with literature data. (A) and (B) show separation results for a 1.8-nm-thick GO membrane, (C) and (D) for 9-nm membrane, and (E) and (F) for an 18-nm membrane. (G) Comparison of ultrathin GO membranes with polymeric membranes and inorganic microporous membranes for H_2/CO_2 mixture separation: selectivity versus H_2 permeance. The black line denotes the 2008 upper bound of the polymeric membrane for H_2/CO_2 (25), assuming membrane thickness is 0.1 μm . Blue points (1 to 9) represent microporous inorganic membranes from the literatures (3, 26–33); red points (10) indicate ultrathin GO membranes from this study. The table at right explains points 1 through 10. ZIF, zeolitic imidazolate framework; MOF, metal-organic framework.

inorganic membranes. Figure S9 shows the comparison of GO membranes with polymeric membranes for H₂/N₂ mixture separation, demonstrating the superior separation performance of GO membranes.

In summary, gas separation membranes, down to 1.8 nm in thickness, were reproducibly fabricated by a facile filtration method. These membranes showed H₂/CO₂ and H₂/N₂ mixture separation selectivities that are one to two orders of magnitude higher than those of the state-of-the-art microporous membranes. The fabrication of membranes on a low-cost polymer support was also demonstrated, making them attractive for the practical H₂ separation from mixtures.

References and Notes

1. Z. P. Lai *et al.*, *Science* **300**, 456–460 (2003).
2. M. Yu, R. D. Noble, J. L. Falconer, *Acc. Chem. Res.* **44**, 1196–1206 (2011).
3. R. M. de Vos, H. Verweij, *Science* **279**, 1710–1711 (1998).
4. M. B. Shiflett, H. C. Foley, *Science* **285**, 1902–1905 (1999).
5. H. B. Park *et al.*, *Science* **318**, 254–258 (2007).
6. D. A. Dikin *et al.*, *Nature* **448**, 457–460 (2007).
7. S. S. Chen *et al.*, *Am. Chem. Soc. Nano* **5**, 1321–1327 (2011).
8. C. Lee, X. D. Wei, J. W. Kysar, J. Hone, *Science* **321**, 385–388 (2008).
9. J. S. Bunch *et al.*, *Nano Lett.* **8**, 2458–2462 (2008).
10. R. R. Nair, H. A. Wu, P. N. Jayaram, I. V. Grigorieva, A. K. Geim, *Science* **335**, 442–444 (2012).
11. O. Leenaerts, B. Partoens, F. M. Peeters, *Appl. Phys. Lett.* **93**, 193107 (2008).
12. S. P. Koenig, L. Wang, J. Pellegrino, J. S. Bunch, *Nat. Nanotechnol.* **7**, 728–732 (2012).
13. See supplementary materials on Science Online.
14. S. T. Oyama, D. Lee, P. Hacıoğlu, R. F. Saraf, *J. Membr. Sci.* **244**, 45–53 (2004).
15. D. E. Jiang, V. R. Cooper, S. Dai, *Nano Lett.* **9**, 4019–4024 (2009).
16. H. L. Du *et al.*, *J. Phys. Chem. C* **115**, 23261–23266 (2011).
17. X. Qin, Q. Y. Meng, Y. P. Feng, Y. F. Gao, *Surf. Sci.* **607**, 153–158 (2013).
18. N. W. Ockwig, T. M. Nenoff, *Chem. Rev.* **107**, 4078–4110 (2007).
19. P. Bernardo, E. Drioli, G. Golemme, *Ind. Eng. Chem. Res.* **48**, 4638–4663 (2009).
20. A. Brunetti, F. Scura, G. Barbieri, E. Drioli, *J. Membr. Sci.* **359**, 115–125 (2010).
21. C. A. Scholes, K. H. Smith, S. E. Kentish, G. W. Stevens, *Int. J. Greenh. Gas Control* **4**, 739–755 (2010).
22. M. Hong, S. G. Li, J. L. Falconer, R. D. Noble, *J. Membr. Sci.* **307**, 277–283 (2008).
23. G. Q. Guan, T. Tanaka, K. Kusakabe, K. I. Sotowa, S. Morooka, *J. Membr. Sci.* **214**, 191–198 (2003).
24. T. Tomita, K. Nakayama, H. Sakai, *Microporous Mesoporous Mater.* **68**, 71–75 (2004).
25. L. M. Robeson, *J. Membr. Sci.* **320**, 390–400 (2008).
26. B. Elyassi, M. Sahimi, T. T. Tsotsis, *J. Membr. Sci.* **288**, 290–297 (2007).
27. M. Hong, J. L. Falconer, R. D. Noble, *Ind. Eng. Chem. Res.* **44**, 4035–4041 (2005).
28. Y. Li, F. Liang, H. Bux, W. Yang, J. Caro, *J. Membr. Sci.* **354**, 48–54 (2010).
29. M. Yu, H. H. Funke, R. D. Noble, J. L. Falconer, *J. Am. Chem. Soc.* **133**, 1748–1750 (2011).
30. Z. Tang, J. Dong, T. M. Nenoff, *Langmuir* **25**, 4848–4852 (2009).
31. M. Kanezashi, J. O'Brien-Abraham, Y. S. Lin, K. Suzuki, *AIChE J.* **54**, 1478–1486 (2008).
32. Y. Gu, S. T. Oyama, *J. Membr. Sci.* **345**, 267–275 (2009).
33. H. Guo, G. Zhu, I. J. Hewitt, S. Qiu, *J. Am. Chem. Soc.* **131**, 1646–1647 (2009).

Acknowledgments: We thank the University of South Carolina for start-up funding, S. Ma for experimental assistance on XPS, C. T. Williams for assistance with Raman spectroscopy, and J. R. Regalbuto for suggestions on writing the manuscript. M.Y. and H.L. are inventors on U.S. patent application number 61/850,415 ("Ultra-thin, Molecular-Sieving Graphene Oxide Membranes for Separations"), applied for by the University of South Carolina.

Supplementary Materials

www.sciencemag.org/content/342/6154/95/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S9

References (34–40)

18 February 2013; accepted 28 August 2013

10.1126/science.1236686

Specific Chemical Reactivities of Spatially Separated 3-Aminophenol Conformers with Cold Ca⁺ Ions

Yuan-Pin Chang,^{1*} Karol Długolecki,¹ Jochen Küpper,^{1,2,3†} Daniel Rösch,^{4*} Dieter Wild,⁴ Stefan Willitsch^{4†}

Many molecules exhibit multiple rotational isomers (conformers) that interconvert thermally and are difficult to isolate. Consequently, a precise characterization of their role in chemical reactions has proven challenging. We have probed the reactivity of specific conformers by using an experimental technique based on their spatial separation in a molecular beam by electrostatic deflection. The separated conformers react with a target of Coulomb-crystallized ions in a trap. In the reaction of Ca⁺ with 3-aminophenol, we find a twofold larger rate constant for the *cis* compared with the *trans* conformer (differentiated by the O–H bond orientation). This result is explained by conformer-specific differences in the long-range ion-molecule interaction potentials. Our approach demonstrates the possibility of controlling reactivity through selection of conformational states.

Recent progress in the cooling, manipulation, and control of isolated molecules in the gas phase (*1–4*) has paved the way

for the study of chemical processes at high levels of sensitivity, selectivity, and detail. Methods for slowing and merging of supersonic molecular beams have enabled precise characterizations of the role of collision energy and of the molecular quantum state in scattering and reactive processes (*5–7*). Recent experiments with trapped, translationally cold molecules and ions have provided insights into the quantum dynamics of chemical reactions (*8*) and the subtleties of intermolecular interactions (*9*). These experiments have thus far been restricted to reactions involving atoms or small molecules with simple geometric and quantum structures. The vast ma-

jority of molecules, however, possess a plethora of internal degrees of freedom that are challenging to probe independently. In particular, polyatomic molecules usually exhibit many rotational isomers (conformers) that interconvert with low thermal barriers through rotations about covalent bonds. Recent years have seen impressive progress in the spectroscopic characterization of specific conformations (*10–12*), their photoinduced isomerization (*13, 14*), and the characterization of conformer-specific photodissociation dynamics (*15, 16*). However, studies of specific conformational effects in bimolecular reactions are still sparse (*17, 18*), in particular in the gas phase, where the highest degree of control and therefore insight into fundamental reaction mechanisms can be gained.

Here, we introduce a distinct approach for the study of conformational effects and conformation-dependent reactivities, that is, rate constants, in bimolecular reactions. Our method exploits the spatial separation of different conformers by using inhomogeneous electric fields (*19, 20*). Internally cold molecules in a beam are dispersed by an electrostatic deflector and are directed toward the reaction volume. There, they interact with laser-cooled ions in a Coulomb crystal, that is, an ordered structure of translationally cold ions in a trap (*4*). The Coulomb crystal constitutes a tightly localized, stationary reaction target much smaller in extension than the conformationally separated regions of the beam so that chemical reactions can be studied selectively with isolated conformers.

We studied the reaction of individual conformers of 3-aminophenol (AP) with Ca⁺ ions to

¹Center for Free-Electron Laser Science, DESY (Deutsches Elektronen-Synchrotron), Notkestrasse 85, 22607 Hamburg, Germany. ²The Hamburg Center for Ultrafast Imaging, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany. ³Department of Physics, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany. ⁴Department of Chemistry, University of Basel, Klingelbergstrasse 80, 4056 Basel, Switzerland.

*These authors contributed equally to this work.

†Corresponding author. E-mail: jochen.kuepper@cfe.de (J.K.); stefan.willitsch@unibas.ch (S.W.)



Specific Chemical Reactivities of Spatially Separated 3-Aminophenol Conformers with Cold Ca^+ Ions

Yuan-Pin Chang *et al.*

Science **342**, 98 (2013);

DOI: 10.1126/science.1242271

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/98.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.98.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/98.full.html#related>

This article **cites 44 articles**, 8 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/98.full.html#ref-list-1>

This article has been **cited by 1** articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/342/6154/98.full.html#related-urls>

inorganic membranes. Figure S9 shows the comparison of GO membranes with polymeric membranes for H₂/N₂ mixture separation, demonstrating the superior separation performance of GO membranes.

In summary, gas separation membranes, down to 1.8 nm in thickness, were reproducibly fabricated by a facile filtration method. These membranes showed H₂/CO₂ and H₂/N₂ mixture separation selectivities that are one to two orders of magnitude higher than those of the state-of-the-art microporous membranes. The fabrication of membranes on a low-cost polymer support was also demonstrated, making them attractive for the practical H₂ separation from mixtures.

References and Notes

1. Z. P. Lai *et al.*, *Science* **300**, 456–460 (2003).
2. M. Yu, R. D. Noble, J. L. Falconer, *Acc. Chem. Res.* **44**, 1196–1206 (2011).
3. R. M. de Vos, H. Verweij, *Science* **279**, 1710–1711 (1998).
4. M. B. Shiflett, H. C. Foley, *Science* **285**, 1902–1905 (1999).
5. H. B. Park *et al.*, *Science* **318**, 254–258 (2007).
6. D. A. Dikin *et al.*, *Nature* **448**, 457–460 (2007).
7. S. S. Chen *et al.*, *Am. Chem. Soc. Nano* **5**, 1321–1327 (2011).
8. C. Lee, X. D. Wei, J. W. Kysar, J. Hone, *Science* **321**, 385–388 (2008).
9. J. S. Bunch *et al.*, *Nano Lett.* **8**, 2458–2462 (2008).
10. R. R. Nair, H. A. Wu, P. N. Jayaram, I. V. Grigorieva, A. K. Geim, *Science* **335**, 442–444 (2012).
11. O. Leenaerts, B. Partoens, F. M. Peeters, *Appl. Phys. Lett.* **93**, 193107 (2008).
12. S. P. Koenig, L. Wang, J. Pellegrino, J. S. Bunch, *Nat. Nanotechnol.* **7**, 728–732 (2012).
13. See supplementary materials on Science Online.
14. S. T. Oyama, D. Lee, P. Hacıoğlu, R. F. Saraf, *J. Membr. Sci.* **244**, 45–53 (2004).
15. D. E. Jiang, V. R. Cooper, S. Dai, *Nano Lett.* **9**, 4019–4024 (2009).
16. H. L. Du *et al.*, *J. Phys. Chem. C* **115**, 23261–23266 (2011).
17. X. Qin, Q. Y. Meng, Y. P. Feng, Y. F. Gao, *Surf. Sci.* **607**, 153–158 (2013).
18. N. W. Ockwig, T. M. Nenoff, *Chem. Rev.* **107**, 4078–4110 (2007).
19. P. Bernardo, E. Drioli, G. Golemme, *Ind. Eng. Chem. Res.* **48**, 4638–4663 (2009).
20. A. Brunetti, F. Scura, G. Barbieri, E. Drioli, *J. Membr. Sci.* **359**, 115–125 (2010).
21. C. A. Scholes, K. H. Smith, S. E. Kentish, G. W. Stevens, *Int. J. Greenh. Gas Control* **4**, 739–755 (2010).
22. M. Hong, S. G. Li, J. L. Falconer, R. D. Noble, *J. Membr. Sci.* **307**, 277–283 (2008).
23. G. Q. Guan, T. Tanaka, K. Kusakabe, K. I. Sotowa, S. Morooka, *J. Membr. Sci.* **214**, 191–198 (2003).
24. T. Tomita, K. Nakayama, H. Sakai, *Microporous Mesoporous Mater.* **68**, 71–75 (2004).
25. L. M. Robeson, *J. Membr. Sci.* **320**, 390–400 (2008).
26. B. Elyassi, M. Sahimi, T. T. Tsotsis, *J. Membr. Sci.* **288**, 290–297 (2007).
27. M. Hong, J. L. Falconer, R. D. Noble, *Ind. Eng. Chem. Res.* **44**, 4035–4041 (2005).
28. Y. Li, F. Liang, H. Bux, W. Yang, J. Caro, *J. Membr. Sci.* **354**, 48–54 (2010).
29. M. Yu, H. H. Funke, R. D. Noble, J. L. Falconer, *J. Am. Chem. Soc.* **133**, 1748–1750 (2011).
30. Z. Tang, J. Dong, T. M. Nenoff, *Langmuir* **25**, 4848–4852 (2009).
31. M. Kanezashi, J. O'Brien-Abraham, Y. S. Lin, K. Suzuki, *AIChE J.* **54**, 1478–1486 (2008).
32. Y. Gu, S. T. Oyama, *J. Membr. Sci.* **345**, 267–275 (2009).
33. H. Guo, G. Zhu, I. J. Hewitt, S. Qiu, *J. Am. Chem. Soc.* **131**, 1646–1647 (2009).

Acknowledgments: We thank the University of South Carolina for start-up funding, S. Ma for experimental assistance on XPS, C. T. Williams for assistance with Raman spectroscopy, and J. R. Regalbutto for suggestions on writing the manuscript. M.Y. and H.L. are inventors on U.S. patent application number 61/850,415 ("Ultra-thin, Molecular-Sieving Graphene Oxide Membranes for Separations"), applied for by the University of South Carolina.

Supplementary Materials

www.sciencemag.org/content/342/6154/95/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S9

References (34–40)

18 February 2013; accepted 28 August 2013

10.1126/science.1236686

Specific Chemical Reactivities of Spatially Separated 3-Aminophenol Conformers with Cold Ca⁺ Ions

Yuan-Pin Chang,^{1*} Karol Długołęcki,¹ Jochen Küpper,^{1,2,3†} Daniel Rösch,^{4*} Dieter Wild,⁴ Stefan Willitsch^{4†}

Many molecules exhibit multiple rotational isomers (conformers) that interconvert thermally and are difficult to isolate. Consequently, a precise characterization of their role in chemical reactions has proven challenging. We have probed the reactivity of specific conformers by using an experimental technique based on their spatial separation in a molecular beam by electrostatic deflection. The separated conformers react with a target of Coulomb-crystallized ions in a trap. In the reaction of Ca⁺ with 3-aminophenol, we find a twofold larger rate constant for the *cis* compared with the *trans* conformer (differentiated by the O–H bond orientation). This result is explained by conformer-specific differences in the long-range ion-molecule interaction potentials. Our approach demonstrates the possibility of controlling reactivity through selection of conformational states.

Recent progress in the cooling, manipulation, and control of isolated molecules in the gas phase (*1–4*) has paved the way

for the study of chemical processes at high levels of sensitivity, selectivity, and detail. Methods for slowing and merging of supersonic molecular beams have enabled precise characterizations of the role of collision energy and of the molecular quantum state in scattering and reactive processes (*5–7*). Recent experiments with trapped, translationally cold molecules and ions have provided insights into the quantum dynamics of chemical reactions (*8*) and the subtleties of intermolecular interactions (*9*). These experiments have thus far been restricted to reactions involving atoms or small molecules with simple geometric and quantum structures. The vast ma-

jority of molecules, however, possess a plethora of internal degrees of freedom that are challenging to probe independently. In particular, polyatomic molecules usually exhibit many rotational isomers (conformers) that interconvert with low thermal barriers through rotations about covalent bonds. Recent years have seen impressive progress in the spectroscopic characterization of specific conformations (*10–12*), their photoinduced isomerization (*13, 14*), and the characterization of conformer-specific photodissociation dynamics (*15, 16*). However, studies of specific conformational effects in bimolecular reactions are still sparse (*17, 18*), in particular in the gas phase, where the highest degree of control and therefore insight into fundamental reaction mechanisms can be gained.

Here, we introduce a distinct approach for the study of conformational effects and conformation-dependent reactivities, that is, rate constants, in bimolecular reactions. Our method exploits the spatial separation of different conformers by using inhomogeneous electric fields (*19, 20*). Internally cold molecules in a beam are dispersed by an electrostatic deflector and are directed toward the reaction volume. There, they interact with laser-cooled ions in a Coulomb crystal, that is, an ordered structure of translationally cold ions in a trap (*4*). The Coulomb crystal constitutes a tightly localized, stationary reaction target much smaller in extension than the conformationally separated regions of the beam so that chemical reactions can be studied selectively with isolated conformers.

We studied the reaction of individual conformers of 3-aminophenol (AP) with Ca⁺ ions to

¹Center for Free-Electron Laser Science, DESY (Deutsches Elektronen-Synchrotron), Notkestrasse 85, 22607 Hamburg, Germany. ²The Hamburg Center for Ultrafast Imaging, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany. ³Department of Physics, University of Hamburg, Luruper Chaussee 149, 22761 Hamburg, Germany. ⁴Department of Chemistry, University of Basel, Klingelbergstrasse 80, 4056 Basel, Switzerland.

*These authors contributed equally to this work.

†Corresponding author. E-mail: jochen.kuepper@cfe.de (J.K.); stefan.willitsch@unibas.ch (S.W.)

probe conformation-dependent reactivities in a prototypical reaction between an organic molecule and a metal ion in the gas phase. Ca^+ was chosen as the ionic reactant because it can be laser-cooled to form Coulomb crystals (4, 21). Its chemical relevance lies in the activation of chemically inert polar bonds (22–24). Reactions of this type are of interest, for example, in the context of catalysis (25, 26), in atmospheric

chemistry (27), and for elucidating interactions between metal ions and biomolecular building blocks (24).

Figure 1 shows a schematic of the experimental setup. The vapor above a sample of AP heated to 145°C was entrained in a pulsed supersonic expansion of neon at a stagnation pressure of 34 bar. AP exhibits two distinct molecular conformations, *cis* and *trans*, that differ in the rela-

tive orientation of the O–H bond with respect to the NH_2 group. The molecular geometries are depicted in Fig. 2A. The barrier to interconversion by rotation of OH about the C–O bond is $\approx 1500 \text{ cm}^{-1}$ (28). Both conformers have significantly different electric dipole moments of 0.77 D and 2.33 D for the *trans* and *cis* species, respectively, giving rise to their distinct Stark interactions with an electric field (29).

In the expansion, the AP molecules were adiabatically cooled to a population ratio of the *cis* and *trans* conformers in the molecular beam of about 1:4 and a rotational temperature of $T_{\text{rot}} = 1.1 \text{ K}$. Thus, over 99% of the population of both conformers was confined to the lowest rotational levels (rotational quantum numbers $j_{\text{AP}} \leq 8$), and practically all molecules were in the vibrational and electronic ground state. In the cold and collisionless environment of the molecular beam, interconversion between the conformers did not occur. After passing two skimmers, the molecular beam entered an electrostatic deflector consisting of a pair of 15-cm-long electrodes to which potential differences in the range of 5 to 13 kV were applied (30). The shape of the electrodes was designed to generate a strong inhomogeneous electric field with a nearly constant gradient along the y axis, as depicted in Fig. 1. For both conformers, the Stark energies of all populated rotational levels decrease with increasing field strength. The molecules were, therefore, deflected toward regions of high electric field in the deflector (20). Because the electric dipole moment is considerably larger for *cis*-AP than for *trans*-AP (29), the *cis* species was more strongly deflected than the *trans* species, resulting in a spatial separation of the two conformers in the beam (20).

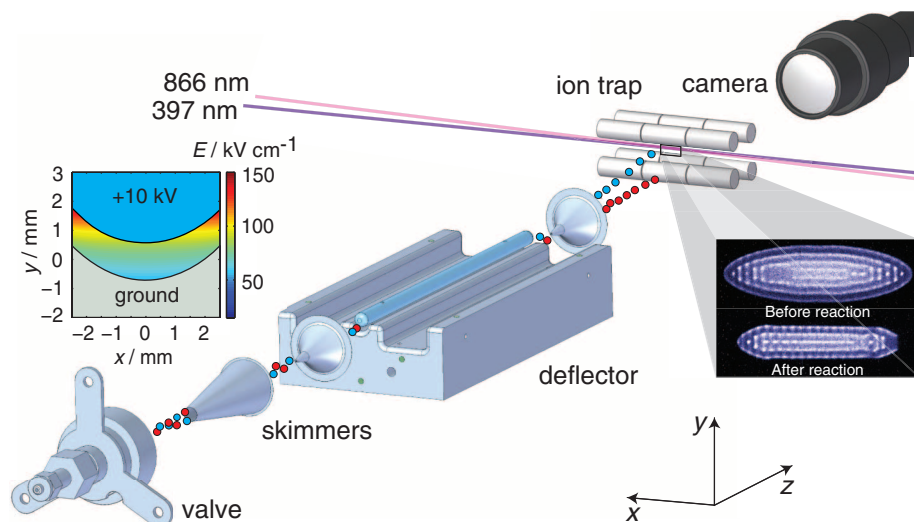


Fig. 1. Experimental setup for studying conformer-selected chemical reactions. *cis*-AP (blue spheres) and *trans*-AP (red spheres) molecules are entrained in a molecular beam. The two conformers are deflected to different extents in the inhomogeneous electric field E (left inset) of an electrostatic deflector. As a consequence, the molecular beam is split into different conformational components. The individual conformers are subsequently aimed at a stationary reaction target comprising a Coulomb crystal of laser-cooled Ca^+ ions in a trap (right inset). Conformer-specific rate constants are determined by monitoring the removal of Ca^+ ions from the Coulomb crystal as a function of the reaction time. The wavelengths of the Ca^+ cooling laser beams are 866 and 397 nm.

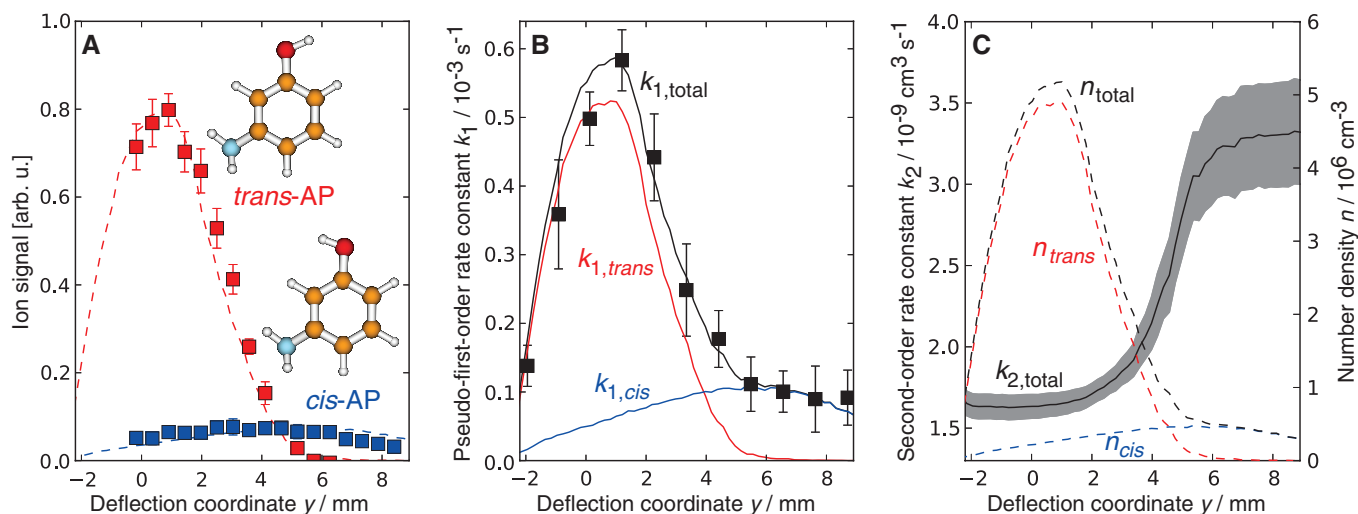


Fig. 2. Reaction kinetics. (A) Density profiles of a deflected beam of *cis*- and *trans*-AP ($V_{\text{defl}} = 7.5 \text{ kV}$) measured by conformer-specific multiphoton ionization as a function of the molecular-beam deflection coordinate y . Squares, experimental data; dashed lines, Monte Carlo trajectory simulations. Arb. u., arbitrary units. (B) Conformer-specific (red and blue) and total (black) pseudo-first-order rate constants k_1 for the reaction $\text{Ca}^+ + \text{cis-/trans-AP}$ as a function of the beam deflection coordinate y at $V_{\text{defl}} = 7.5 \text{ kV}$. (C) Total bi-

molecular rate constant $k_{2,\text{total}}(y)$ (black solid line) illustrating the increase in chemical reactivity as the predominant beam component changes from the *trans* to the *cis* conformer. Dashed lines, number densities of the two conformers. The data points represent the statistical averages of 1000 laser shots in (A) and a minimum of four reaction measurements in (B). Error bars indicate the corresponding 95% confidence intervals. The gray area in (C) represents the 95% confidence interval of $k_{2,\text{total}}$.

Figure 2A shows density profiles, $n_{\text{cis}}(y)$ and $n_{\text{trans}}(y)$, for the deflected *cis*- and *trans*-AP molecules, respectively. The profiles were obtained by measuring the ion signal produced by conformer-selective resonance-enhanced multiphoton ionization (REMPI) (20) as a function of the vertical deflection coordinate when applying a potential difference of $V_{\text{defl}} = 7.5$ kV to the deflector electrodes. The molecular beam assembly was tilted incrementally in the vertical direction in order to scan the deflected molecular beam over the position of the fixed ionization laser spot 82 cm downstream from the exit of the deflector. The deflection coordinate y in Fig. 2 is defined as the vertical displacement of the center of the nominally undeflected beam from the interaction point. The dashed lines represent the results of Monte Carlo trajectory simulations (21). In the calculations, the rotational temperature of the molecules and one global scaling factor were adjusted to optimally reproduce the experimental data. At low deflection coordinates the *trans* conformation dominates, whereas at the highest deflection coordinates only the *cis* conformer is present.

For the reaction experiments, the REMPI spectrometer was replaced by a linear quadrupole ion trap for the generation of Coulomb crystals of laser-cooled Ca^+ ions (4, 21) (Fig. 1). The Coulomb crystals, typically consisting of 700 ions, were imaged by a camera sampling the atomic fluorescence generated by the laser cooling of the ions. The Ca^+ ions were exposed to AP molecules from the deflected molecular beam. Because the AP molecules in the reaction volume were replenished with each gas pulse, their number density was essentially constant over the measurement time. Thus, pseudo-first-order reaction rate constants could be determined from the decrease of the number of Ca^+ ions in the crystals (21, 31). Product ions formed in the reaction remained confined in the trap. They were sympathetically cooled by the interaction with the remaining laser-cooled Ca^+ ions and localized at the edges of the Coulomb crystals (4). These ions did not fluoresce and were only indirectly visible in the images through a characteristic flattening of the Ca^+ crystal edges (32), as shown in Fig. 1.

The products of the reaction were analyzed by using resonant-excitation mass spectrometry of the ions in the trap, suggesting CaOH^+ or CaNH_2^+ ions and the corresponding 3-aminophenyl or 3-hydroxyphenyl radicals as the primary reaction products (21). In the experiment, the $(4s) {}^2S_{1/2}$, $(4p) {}^2P_{1/2}$, and $(3d) {}^2D_{3/2}$ states of Ca^+ were populated as a consequence of the laser cooling. The rate constant for reactions out of the excited $(4p) {}^2P_{1/2}$ state was found to be two to three orders of magnitude larger than the rate coefficients for reactions out of the two other states, so this process dominated the effective rates measured in the present study (21). In the following, we focus on the $\text{Ca}^+(4p) + \text{AP}$ reaction, and all second-order rate constants quoted below refer to this particular channel.

Figure 2B shows the total effective pseudo-first-order rate constant $k_{1,\text{total}}$ as a function of the deflection coordinate y . The measured rate constant profile $k_{1,\text{total}}(y)$ reflects the density distribution of the conformers in the deflected molecular beam as well as the conformer-specific second-order rate constants $k_{2,\text{cis}}$ and $k_{2,\text{trans}}$ for the reactions of *cis*-AP and *trans*-AP, respectively, with $\text{Ca}^+(4p)$

$$k_{1,\text{total}}(y) = k_{2,\text{cis}}p_{4p}n_{\text{cis}}(y) + k_{2,\text{trans}}p_{4p}n_{\text{trans}}(y) \quad (1)$$

p_{4p} is the population in the $\text{Ca}^+(4p)$ state (21). $k_{2,\text{cis}}$ and $k_{2,\text{trans}}$ were determined from a global fit of Eq. 1 to reaction-rate profiles obtained at deflector voltages $V_{\text{defl}} = 5, 7.5, 10$, and 13 kV. The fit yielded the rate constants $k_{2,\text{cis}} = 3.2 \times 10^{-9} \pm 1.3 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ and $k_{2,\text{trans}} = 1.5 \times 10^{-9} \pm 0.6 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ and the ratio $k_{2,\text{cis}}/k_{2,\text{trans}} = 2.1 \pm 0.5$ within a 95% confidence interval. Figure 2C shows the total second-order rate constant $k_{2,\text{total}}(y) = x_{\text{cis}}(y)k_{2,\text{cis}} + x_{\text{trans}}(y)k_{2,\text{trans}}$ obtained from the fit, where $x_{\text{cis}}(y)$ and $x_{\text{trans}}(y)$ denote the mole fractions of *cis*-AP and *trans*-AP in the molecular beam, respectively. This plot highlights the change of the reaction rate as the beam composition evolves from *trans* to *cis*.

$k_{2,\text{cis}}$ and $k_{2,\text{trans}}$ are of similar magnitude as the rate constants typically obtained for ion-molecule reactions with capture-limited kinetics (33). In these cases, the reaction rates are dominated by long-range intermolecular interactions in the entrance channel and are independent of the details of the short-range reaction mechanism. The rate constants can then appropriately be modeled by using adiabatic capture models (34–36). These models assume that the reaction happens with unit probability if the collision energy exceeds the height of the centrifugal barrier in the entrance channel at a given value of the total angular momentum quantum number J . Figure 3, A and B, shows the centrifugally corrected long-range potentials for different values of J in the entrance channel of the reaction of Ca^+ with *cis*- and *trans*-AP, respectively. The potentials have been constructed from the charge-dipole and the charge-

induced dipole interactions, which represent the dominant long-range forces relevant for the collisions (21, 35).

Because of the larger dipole moment of the *cis* conformer, the interaction potential is more strongly attractive for *cis*-AP than for *trans*-AP. This can directly be seen from a comparison of the $J = 0$ potentials of the *cis* and *trans* conformers in Fig. 3, A and B, respectively. As a result, the centrifugal barrier is more strongly suppressed in the reaction with *cis*-AP so that reactive collisions can occur up to higher maximum values J_{max} of J . At the collision energy of the experiment, we find $J_{\text{max}} = 417$ and 342 for the *cis* and *trans* conformers, respectively. Hence, in a classical picture the *cis* conformer exhibits a larger maximum impact parameter $b_{\text{max}} = J_{\text{max}}/\mu v$ for the reaction (where μ is the reduced mass and v the collision velocity) and, therefore, an increased reaction cross section $\sigma = \pi b_{\text{max}}^2$. Averaging over all populated rotational states, the adiabatic capture model predicts the rate constants $k_{2,\text{cis}} = 2.7 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ and $k_{2,\text{trans}} = 1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$. The ratio of the theoretical rate constants is calculated to be $k_{2,\text{cis}}/k_{2,\text{trans}} = 1.5$ in agreement with the experimental results. Moreover, the absolute values of these capture rate constants are in very good agreement with the experimentally obtained values.

Thus, in the present case the increased reaction rate for the *cis* species can be traced back to its increased collision rate with Ca^+ . This effect results from conformation-dependent electrostatic properties of the molecules and the resulting differences in the long-range interaction potentials. Short-range conformational effects play only a minor role, as discussed in the supplementary text.

The isolation of individual conformers opens new avenues to control the outcome of chemical reactions by selecting conformer-specific pathways that result in different product distributions. This capability has already been demonstrated in previous conformationally resolved photodissociation experiments (15, 16) and is now within reach for gas-phase reaction experiments.

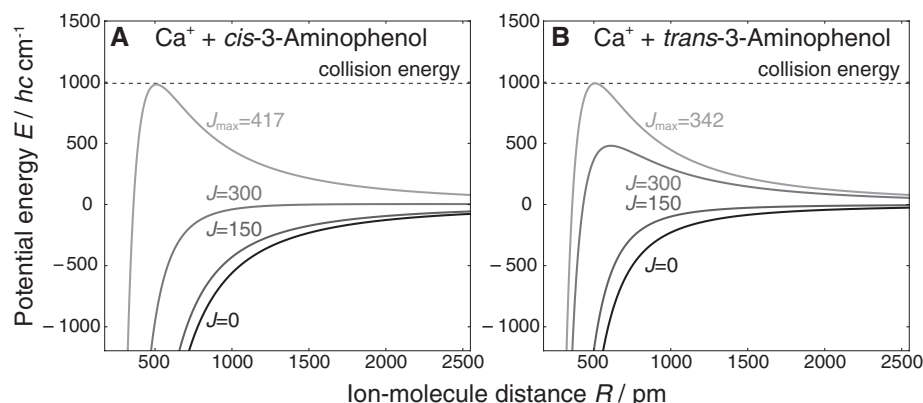


Fig. 3. Centrifugally corrected potential energy curves for rotationless (A) *cis*- and (B) *trans*-AP molecules reacting with Ca^+ .

The present advances have become possible through the combination of electrostatic conformer selection with highly sensitive Coulomb-crystal methods. We expect that the current methodology will benefit fundamental reaction-dynamics studies as well as the investigation of a wide range of ion-molecule reactions with relevance for catalysis and interstellar chemistry. Electrostatic conformer selection is a widely applicable technique whenever the conformers present in the molecular beam possess sufficiently different dipole moments. Even more advanced electric field manipulation techniques for the separation of individual chemical species (19, 37) or individual quantum states (1, 38) have been demonstrated. In addition, sympathetic cooling of ions is a near-universal technique that allows the generation of Coulomb crystals of a wide range of atomic and molecular species with simultaneous preparation of their internal quantum state (4, 39).

References and Notes

1. S. Y. T. van de Meerakker, H. L. Bethlem, N. Vanhaecke, G. Meijer, *Chem. Rev.* **112**, 4828–4878 (2012).
2. G. Quémener, P. S. Julienne, *Chem. Rev.* **112**, 4949–5011 (2012).
3. F. Filsinger, G. Meijer, H. Stapelfeldt, H. N. Chapman, J. Küpper, *Phys. Chem. Chem. Phys.* **13**, 2076–2087 (2011).
4. S. Willitsch, *Int. Rev. Phys. Chem.* **31**, 175–199 (2012).
5. J. J. Gilijamse, S. Hoekstra, S. Y. T. van de Meerakker, G. C. Groenenboom, G. Meijer, *Science* **313**, 1617–1620 (2006).
6. M. Kirste *et al.*, *Science* **338**, 1060–1063 (2012).
7. A. B. Henson, S. Gersten, Y. Shagam, J. Narevicius, E. Narevicius, *Science* **338**, 234–238 (2012).
8. S. Ospelkaus *et al.*, *Science* **327**, 853–857 (2010).
9. F. H. J. Hall, S. Willitsch, *Phys. Rev. Lett.* **109**, 233202 (2012).
10. J. P. Simons *et al.*, *Int. Rev. Phys. Chem.* **24**, 489–531 (2005).
11. M. S. de Vries, P. Hobza, *Annu. Rev. Phys. Chem.* **58**, 585–612 (2007).
12. T. R. Rizzo, J. A. Stearns, O. V. Boyarkin, *Int. Rev. Phys. Chem.* **28**, 481–515 (2009).
13. B. C. Dian, J. R. Clarkson, T. S. Zwier, *Science* **303**, 1169–1173 (2004).
14. B. C. Dian, G. G. Brown, K. O. Douglass, B. H. Pate, *Science* **320**, 924–928 (2008).
15. S. T. Park, S. K. Kim, M. S. Kim, *Nature* **415**, 306–308 (2002).
16. M. H. Kim, L. Shen, H. Tao, T. J. Martinez, A. G. Suits, *Science* **315**, 1561–1565 (2007).
17. L. Khriachtchev *et al.*, *J. Phys. Chem. A* **113**, 8143–8146 (2009).
18. C. A. Taatjes *et al.*, *Science* **340**, 177–180 (2013).
19. F. Filsinger, U. Erlekam, G. von Helden, J. Küpper, G. Meijer, *Phys. Rev. Lett.* **100**, 133003 (2008).
20. F. Filsinger *et al.*, *Angew. Chem. Int. Ed.* **48**, 6900–6902 (2009).
21. Materials and methods are available as supplementary materials on Science Online.
22. J. N. Harvey *et al.*, *Chem. Phys. Lett.* **278**, 391–397 (1997).
23. X. Zhao, G. K. Koyanagi, D. K. Bohme, *J. Phys. Chem. A* **110**, 10607–10618 (2006).
24. V. Ryzhov, R. C. Dunbar, *J. Am. Chem. Soc.* **121**, 2259–2268 (1999).
25. K. Eller, H. Schwarz, *Chem. Rev.* **91**, 1121–1177 (1991).
26. H. Schwarz, *Angew. Chem. Int. Ed.* **50**, 10096–10115 (2011).
27. S. L. Broadley, T. Vondrak, J. M. C. Plane, *Phys. Chem. Chem. Phys.* **9**, 4357–4369 (2007).
28. T. W. Robinson, H. G. Kjaergaard, S.-I. Ishiuchi, M. Shinozaki, M. Fujii, *J. Phys. Chem. A* **108**, 4420–4427 (2004).
29. F. Filsinger, K. Wohlfart, M. Schnell, J.-U. Grabow, J. Küpper, *Phys. Chem. Chem. Phys.* **10**, 666–673 (2008).
30. F. Filsinger *et al.*, *J. Chem. Phys.* **131**, 064309 (2009).
31. S. Willitsch, M. T. Bell, A. D. Gingell, S. R. Procter, T. P. Softley, *Phys. Rev. Lett.* **100**, 043203 (2008).
32. S. Willitsch, M. T. Bell, A. D. Gingell, T. P. Softley, *Phys. Chem. Chem. Phys.* **10**, 7200–7210 (2008).
33. B. R. Rowe, J. B. Marquette, *Int. J. Mass Spectrom. Ion Process.* **80**, 239–254 (1987).
34. D. C. Clary, *Mol. Phys.* **54**, 605–618 (1985).
35. T. Stoecklin, D. C. Clary, A. Palma, *J. Chem. Soc. Faraday Trans.* **88**, 901–908 (1992).
36. J. Troe, *J. Chem. Phys.* **87**, 2773–2780 (1987).
37. S. Trippel *et al.*, *Phys. Rev. A* **86**, 033202 (2012).
38. J. H. Nielsen *et al.*, *Phys. Chem. Chem. Phys.* **13**, 18971–18975 (2011).
39. X. Tong, A. H. Winney, S. Willitsch, *Phys. Rev. Lett.* **105**, 143001 (2010).

Acknowledgments: This work has been supported by the excellence cluster “The Hamburg Center for Ultrafast Imaging—Structure, Dynamics and Control of Matter at the Atomic Scale” of the Deutsche Forschungsgemeinschaft, the Swiss National Science Foundation grant no. PP00P2_140834, and the University of Basel.

Supplementary Materials

www.sciencemag.org/content/342/6154/98/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S3

Table S1

References (40–47)

21 June 2013; accepted 26 August 2013

10.1126/science.1242271

Nitrogen Isotopic Composition and Density of the Archean Atmosphere

Bernard Marty,^{1*} Laurent Zimmermann,¹ Magali Pujol,^{1†} Ray Burgess,² Pascal Philippot³

Understanding the atmosphere’s composition during the Archean eon is fundamental to unraveling ancient environmental conditions. We show from the analysis of nitrogen and argon isotopes in fluid inclusions trapped in 3.0- to 3.5-billion-year-old hydrothermal quartz that the partial pressure of N₂ of the Archean atmosphere was lower than 1.1 bar, possibly as low as 0.5 bar, and had a nitrogen isotopic composition comparable to the present-day one. These results imply that dinitrogen did not play a significant role in the thermal budget of the ancient Earth and that the Archean partial pressure of CO₂ was probably lower than 0.7 bar.

Nitrogen is a key element of planetary atmospheres that is a sensitive tracer of exchanges of volatile elements between

planetary interiors and outer space (1–3). On Earth, the amount of N at Earth’s surface might have varied significantly with time, as a result of exchange with the deep Earth (1, 4) or of loss to space (3, 5). In contrast, the amount of non-radiogenic (not produced by nuclear reactions) noble gas isotopes in the terrestrial atmosphere is unlikely to have varied significantly since Earth’s formation (6). Calibrating N against a nonradiogenic noble isotope such as ³⁶Ar in ancient samples provides a way to estimate the variations of the partial pressure of atmospheric N₂ (P_{N₂}) in the past.

We estimate here the P_{N₂} in the ancient terrestrial atmosphere from the analysis of the N₂/³⁶Ar

ratios in well-characterized fluid inclusions trapped in hydrothermal quartz from the Archean 3.49-billion-year-old (Gy-old) Dresser (7–10) and 3.46-Gy-old Apex Basalt (11, 12) formations in Pilbara Craton, Western Australia. Previous geochemical studies of fluid inclusions from this area (9, 11–16) and from other geological settings (17) have shown the presence of a paleo-atmospheric component, probably incorporated as atmospheric gases dissolved in surface waters. The abundances of atmospheric gases in water are a direct function of their respective partial pressures and of the salinity and temperature of water (18). For the present-day atmospheric partial pressures of N₂ (0.7906 bar) and ³⁶Ar (3.20 × 10^{−5} bar), the N₂/³⁶Ar ratio of air-saturated water (ASW) ranges from 1.02 × 10⁴ to 1.31 × 10⁴, for water temperatures between 2°C (the present-day average deep-sea temperature) and 70°C [a proposed model temperature for the Archean oceans (19)] and salinities between 0 and 16 weight % equivalent of NaCl [encompassing the range of salinities observed in Archean Dresser and Apex fluid inclusions (9, 11, 12)]. Nishizawa *et al.* (13) have shown that, in fluid inclusions trapped in silica dykes and quartz veins from the Dresser Formation, N₂ is the main N species, far more abundant than the other identified species (NH₄⁺). These authors proposed an upper limit of 3.3 times the modern value for the Archean N₂/³⁶Ar ratio.

¹Centre de Recherches Pétrographiques et Géochimiques–CNRS, Université de Lorraine, 15 rue Notre Dame des Pauvres, 54501 Vandœuvre-lès-Nancy Cedex, France. ²School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK. ³Institut de Physique du Globe de Paris, Sorbonne-Paris Cité, Université Paris Diderot, CNRS, 1 rue Jussieu, 75238 Paris Cedex 5, France.

*Corresponding author. E-mail: bmarty@crpg.cnrs-nancy.fr

†Present address: Fluid and Organic Geochemistry, TOTAL Exploration and Production, Avenue Larribau, 64000 PAU, France.



Nitrogen Isotopic Composition and Density of the Archean Atmosphere

Bernard Marty *et al.*

Science **342**, 101 (2013);

DOI: 10.1126/science.1240971

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/101.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/09/19/science.1240971.DC2.html>

<http://www.sciencemag.org/content/suppl/2013/09/18/science.1240971.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/101.full.html#related>

This article **cites 33 articles**, 2 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/101.full.html#ref-list-1>

The present advances have become possible through the combination of electrostatic conformer selection with highly sensitive Coulomb-crystal methods. We expect that the current methodology will benefit fundamental reaction-dynamics studies as well as the investigation of a wide range of ion-molecule reactions with relevance for catalysis and interstellar chemistry. Electrostatic conformer selection is a widely applicable technique whenever the conformers present in the molecular beam possess sufficiently different dipole moments. Even more advanced electric field manipulation techniques for the separation of individual chemical species (19, 37) or individual quantum states (1, 38) have been demonstrated. In addition, sympathetic cooling of ions is a near-universal technique that allows the generation of Coulomb crystals of a wide range of atomic and molecular species with simultaneous preparation of their internal quantum state (4, 39).

References and Notes

1. S. Y. T. van de Meerakker, H. L. Bethlem, N. Vanhaecke, G. Meijer, *Chem. Rev.* **112**, 4828–4878 (2012).
2. G. Quémener, P. S. Julienne, *Chem. Rev.* **112**, 4949–5011 (2012).
3. F. Filsinger, G. Meijer, H. Stapelfeldt, H. N. Chapman, J. Küpper, *Phys. Chem. Chem. Phys.* **13**, 2076–2087 (2011).
4. S. Willitsch, *Int. Rev. Phys. Chem.* **31**, 175–199 (2012).
5. J. J. Gilijamse, S. Hoekstra, S. Y. T. van de Meerakker, G. C. Groenenboom, G. Meijer, *Science* **313**, 1617–1620 (2006).
6. M. Kirste *et al.*, *Science* **338**, 1060–1063 (2012).
7. A. B. Henson, S. Gersten, Y. Shagam, J. Narevicius, E. Narevicius, *Science* **338**, 234–238 (2012).
8. S. Ospelkaus *et al.*, *Science* **327**, 853–857 (2010).
9. F. H. J. Hall, S. Willitsch, *Phys. Rev. Lett.* **109**, 233202 (2012).
10. J. P. Simons *et al.*, *Int. Rev. Phys. Chem.* **24**, 489–531 (2005).
11. M. S. de Vries, P. Hobza, *Annu. Rev. Phys. Chem.* **58**, 585–612 (2007).
12. T. R. Rizzo, J. A. Stearns, O. V. Boyarkin, *Int. Rev. Phys. Chem.* **28**, 481–515 (2009).
13. B. C. Dian, J. R. Clarkson, T. S. Zwier, *Science* **303**, 1169–1173 (2004).
14. B. C. Dian, G. G. Brown, K. O. Douglass, B. H. Pate, *Science* **320**, 924–928 (2008).
15. S. T. Park, S. K. Kim, M. S. Kim, *Nature* **415**, 306–308 (2002).
16. M. H. Kim, L. Shen, H. Tao, T. J. Martinez, A. G. Suits, *Science* **315**, 1561–1565 (2007).
17. L. Khriachtchev *et al.*, *J. Phys. Chem. A* **113**, 8143–8146 (2009).
18. C. A. Taatjes *et al.*, *Science* **340**, 177–180 (2013).
19. F. Filsinger, U. Erlekam, G. von Helden, J. Küpper, G. Meijer, *Phys. Rev. Lett.* **100**, 133003 (2008).
20. F. Filsinger *et al.*, *Angew. Chem. Int. Ed.* **48**, 6900–6902 (2009).
21. Materials and methods are available as supplementary materials on Science Online.
22. J. N. Harvey *et al.*, *Chem. Phys. Lett.* **278**, 391–397 (1997).
23. X. Zhao, G. K. Koyanagi, D. K. Bohme, *J. Phys. Chem. A* **110**, 10607–10618 (2006).
24. V. Ryzhov, R. C. Dunbar, *J. Am. Chem. Soc.* **121**, 2259–2268 (1999).
25. K. Eller, H. Schwarz, *Chem. Rev.* **91**, 1121–1177 (1991).
26. H. Schwarz, *Angew. Chem. Int. Ed.* **50**, 10096–10115 (2011).
27. S. L. Broadley, T. Vondrak, J. M. C. Plane, *Phys. Chem. Chem. Phys.* **9**, 4357–4369 (2007).
28. T. W. Robinson, H. G. Kjaergaard, S.-I. Ishiuchi, M. Shinozaki, M. Fujii, *J. Phys. Chem. A* **108**, 4420–4427 (2004).
29. F. Filsinger, K. Wohlfart, M. Schnell, J.-U. Grabow, J. Küpper, *Phys. Chem. Chem. Phys.* **10**, 666–673 (2008).
30. F. Filsinger *et al.*, *J. Chem. Phys.* **131**, 064309 (2009).
31. S. Willitsch, M. T. Bell, A. D. Gingell, S. R. Procter, T. P. Softley, *Phys. Rev. Lett.* **100**, 043203 (2008).
32. S. Willitsch, M. T. Bell, A. D. Gingell, T. P. Softley, *Phys. Chem. Chem. Phys.* **10**, 7200–7210 (2008).
33. B. R. Rowe, J. B. Marquette, *Int. J. Mass Spectrom. Ion Process.* **80**, 239–254 (1987).
34. D. C. Clary, *Mol. Phys.* **54**, 605–618 (1985).
35. T. Stoecklin, D. C. Clary, A. Palma, *J. Chem. Soc. Faraday Trans.* **88**, 901–908 (1992).
36. J. Troe, *J. Chem. Phys.* **87**, 2773–2780 (1987).
37. S. Trippel *et al.*, *Phys. Rev. A* **86**, 033202 (2012).
38. J. H. Nielsen *et al.*, *Phys. Chem. Chem. Phys.* **13**, 18971–18975 (2011).
39. X. Tong, A. H. Winney, S. Willitsch, *Phys. Rev. Lett.* **105**, 143001 (2010).

Acknowledgments: This work has been supported by the excellence cluster “The Hamburg Center for Ultrafast Imaging—Structure, Dynamics and Control of Matter at the Atomic Scale” of the Deutsche Forschungsgemeinschaft, the Swiss National Science Foundation grant no. PP00P2_140834, and the University of Basel.

Supplementary Materials

www.sciencemag.org/content/342/6154/98/suppl/DC1

Materials and Methods

Supplementary Text

Figs. S1 to S3

Table S1

References (40–47)

21 June 2013; accepted 26 August 2013

10.1126/science.1242271

Nitrogen Isotopic Composition and Density of the Archean Atmosphere

Bernard Marty,^{1*} Laurent Zimmermann,¹ Magali Pujol,^{1†} Ray Burgess,² Pascal Philippot³

Understanding the atmosphere’s composition during the Archean eon is fundamental to unraveling ancient environmental conditions. We show from the analysis of nitrogen and argon isotopes in fluid inclusions trapped in 3.0- to 3.5-billion-year-old hydrothermal quartz that the partial pressure of N₂ of the Archean atmosphere was lower than 1.1 bar, possibly as low as 0.5 bar, and had a nitrogen isotopic composition comparable to the present-day one. These results imply that dinitrogen did not play a significant role in the thermal budget of the ancient Earth and that the Archean partial pressure of CO₂ was probably lower than 0.7 bar.

Nitrogen is a key element of planetary atmospheres that is a sensitive tracer of exchanges of volatile elements between

planetary interiors and outer space (1–3). On Earth, the amount of N at Earth’s surface might have varied significantly with time, as a result of exchange with the deep Earth (1, 4) or of loss to space (3, 5). In contrast, the amount of non-radiogenic (not produced by nuclear reactions) noble gas isotopes in the terrestrial atmosphere is unlikely to have varied significantly since Earth’s formation (6). Calibrating N against a nonradiogenic noble isotope such as ³⁶Ar in ancient samples provides a way to estimate the variations of the partial pressure of atmospheric N₂ (P_{N_2}) in the past.

We estimate here the P_{N_2} in the ancient terrestrial atmosphere from the analysis of the N₂/³⁶Ar

ratios in well-characterized fluid inclusions trapped in hydrothermal quartz from the Archean 3.49-billion-year-old (Gy-old) Dresser (7–10) and 3.46-Gy-old Apex Basalt (11, 12) formations in Pilbara Craton, Western Australia. Previous geochemical studies of fluid inclusions from this area (9, 11–16) and from other geological settings (17) have shown the presence of a paleo-atmospheric component, probably incorporated as atmospheric gases dissolved in surface waters. The abundances of atmospheric gases in water are a direct function of their respective partial pressures and of the salinity and temperature of water (18). For the present-day atmospheric partial pressures of N₂ (0.7906 bar) and ³⁶Ar (3.20 × 10^{−5} bar), the N₂/³⁶Ar ratio of air-saturated water (ASW) ranges from 1.02 × 10⁴ to 1.31 × 10⁴, for water temperatures between 2°C (the present-day average deep-sea temperature) and 70°C [a proposed model temperature for the Archean oceans (19)] and salinities between 0 and 16 weight % equivalent of NaCl [encompassing the range of salinities observed in Archean Dresser and Apex fluid inclusions (9, 11, 12)]. Nishizawa *et al.* (13) have shown that, in fluid inclusions trapped in silica dykes and quartz veins from the Dresser Formation, N₂ is the main N species, far more abundant than the other identified species (NH₄⁺). These authors proposed an upper limit of 3.3 times the modern value for the Archean N₂/³⁶Ar ratio.

¹Centre de Recherches Pétrographiques et Géochimiques–CNRS, Université de Lorraine, 15 rue Notre Dame des Pauvres, 54501 Vandœuvre-lès-Nancy Cedex, France. ²School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK. ³Institut de Physique du Globe de Paris, Sorbonne-Paris Cité, Université Paris Diderot, CNRS, 1 rue Jussieu, 75238 Paris Cedex 5, France.

*Corresponding author. E-mail: bmarty@crpg.cnrs-nancy.fr

†Present address: Fluid and Organic Geochemistry, TOTAL Exploration and Production, Avenue Larribau, 64000 PAU, France.

Fluid inclusions trapped in three different Archean hydrothermal quartz samples were analyzed (9–12, 15, 16). The choice of the samples was driven by the goal of targeting pristine fluids of marine and/or meteoritic origin. Ideally, the best samples to be investigated would be sedimentary rocks. However, precipitated minerals, such as carbonates, sulfates, and halides, are relatively weak mineral phases as compared to quartz, seldom preserving primary fluid inclusions. Silicified sediments were another option, but the grain size is generally too small to preserve fluid inclusions large enough to analyze (several micrometers). For this reason, we chose to investigate quartz-bearing amygdules preserved in komatiitic basalt flow (sample PI-06) at the base of the Dresser Formation and recovered through drilling, and quartz-bearing pods filling retreat voids in exposed pillow basalts (PI-02-39) at the top of the Dresser Formation and within the Apex Formation in the Warranoowa syncline (PB-02-122). Considering the high silica content of Archean seawater and hydrothermal fluids, such voids were probably filled with quartz immediately upon cooling. The fact that the intrapillow pods form well-defined ovoid shapes isolated in the core of the pillow indicates that fluid circulation processes driving mineral precipitation should have occurred through a porous medium shortly after basalt deposition. In order to maximize

sampling of primary fluids of surficial origin (seawater and meteoritic) and to minimize the imprint of hydrothermal fluids, all samples were collected in undeformed rocks located in close proximity to the overlying marine/lacustrine sediments. This, together with the shallow-water character of both the Dresser and Apex settings, indicates that the samples investigated contain fluids of marine and/or lacustrine origin, in addition to hydrothermal fluids.

The PI-06 quartz sample is from a drill core (Pilbara Drilling Project 2) in the Dresser formation and was selected in the depth interval from 102 to 110 m. The quartz fills 2- to 10-mm vesicles in komatiitic basalt at the base of the Dresser Formation and contains 2- to 10- μ m-sized fluid inclusions. The formation of quartz in amygdules in komatiitic basalt must have occurred early in the lava post-emplacement history, because neither deformation nor pressure tracks were observed (15). Fluids trapped in the inclusions have been interpreted as being a mixture of Archean surface water and hydrothermal fluid (15, 20). Ar-Ar analysis of trapped fluids indicates that they are $\geq 3.0 \pm 0.2$ Gy old (15) and that they contain inherited ^{40}Ar , presumably from a hydrothermal end member.

Samples PI-02-39 and PB-02-122 are from isolated quartz-carbonate aggregates forming pods hosted in pillow basalts now exposed at

the surface. These pods resemble typical mineralization structures associated with passive hydrothermal circulation of water through shallow crust. Intra-pillow quartz crystals, which were selected for analysis, contain abundant, 1- to 25- μ m, two-phase (liquid and $<5\%$ CO_2 vapor) aqueous inclusions (9). Fluid inclusions are randomly distributed throughout the host quartz, which argues for a primary origin. The absence of crosscutting veins, metamorphic overprint, and deformation features affecting pillow basalts and associated pods indicates negligible fluid remobilization and circulation after deposition and crystallization. Foriel *et al.* (9) demonstrated that several fluids were trapped in PI-02-39, such as an evolved Archean water component and several (Ba- and Fe-rich) fluid components. A previous noble gas (Ar and Xe) study of sample PI-02-39 (16) has shown that the quartz has preserved, since the meso-Archean era, a paleoatmospheric component having a $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 143 ± 24 , mixed with an hydrothermal fluid component rich in Cl, K, and inherited ^{40}Ar . The Ar-Ar analysis of PI-02-39 indicates that fluids are >2.7 Gy old, probably contemporaneous to the deposition of the Dresser formation (16). Although not investigated in detail for its fluid inclusion composition, sample PB-02-122 shows the same type of fluid inclusion distribution and texture as sample PI-02-39. Further sample char-

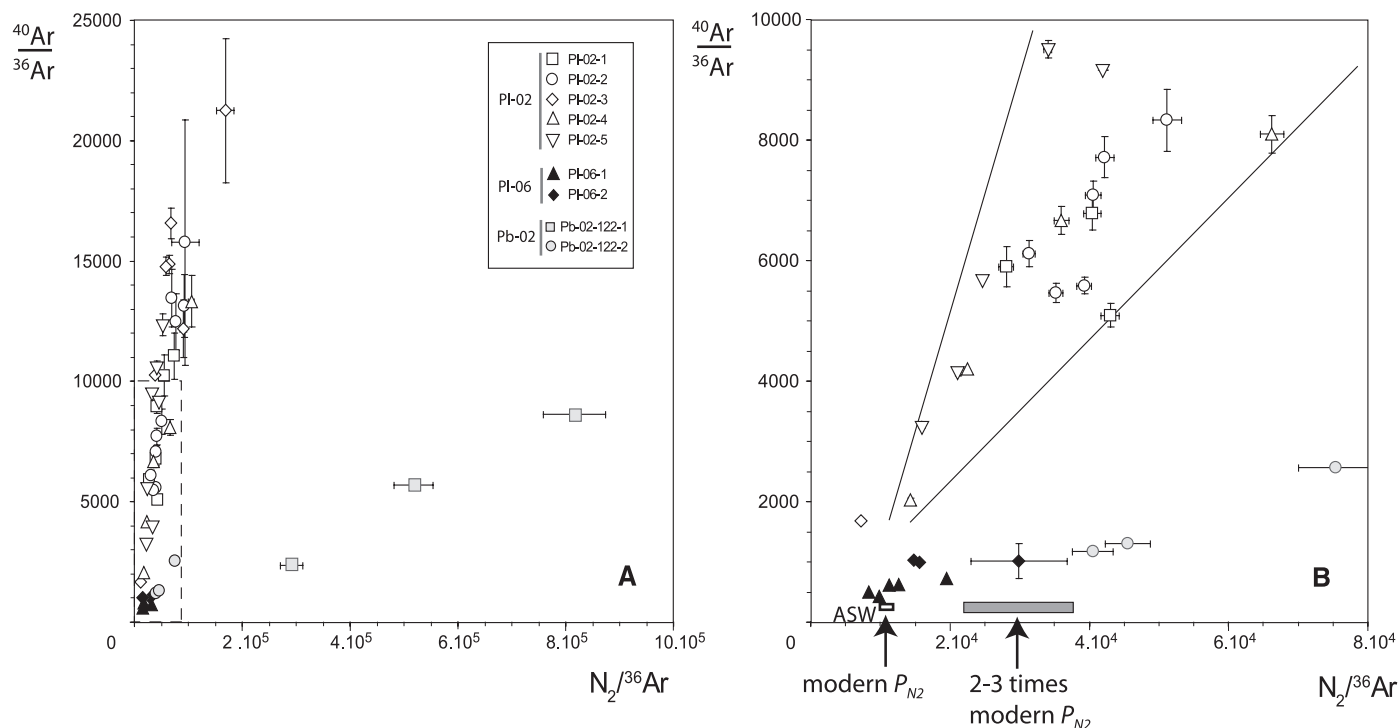


Fig. 1. N-Ar isotope variations for inclusion fluids trapped in Archean quartz. Open, black, and gray symbols are duplicate step-crushing runs for samples PI-02-39, PI-06, and PB-02-122, respectively. (B) is an enlargement of the zone outlined with dashes in (A). The lines in (B) represent upper and lower limits for sample PI-02-39 data points, which converge toward ASW. This sample contains a mixture of several hydrothermal fluids having different $\text{N}_2/^{36}\text{Ar}$ compositions, with a Cl-poor water component. ASW:

air-saturated water with $\text{N}_2/^{36}\text{Ar}$ values corresponding to modern atmospheric partial pressures of N and Ar (table S3) and with $^{40}\text{Ar}/^{36}\text{Ar}$ ratios <298 (the modern value). The corresponding modern partial pressure of atmospheric N_2 is also indicated. A two to three times higher N_2 pressure, as suggested by (1, 2), would correspond to the gray bar on the right-hand side of ASW. Such a higher value is not supported by the convergence of data point correlations.

acteristics, field locations, and photos are given in the supplementary materials (20).

Several aliquots of each sample were crushed under vacuum using different numbers of crushing steps, and the extracted gases were sequentially analyzed for N and Ar isotopic abundances by static mass spectrometry (table S1). For all runs, the N and Ar abundances correlate, showing that it is unlikely that much N in the fluid inclusions has been consumed by postentrapment chemical reactions. Before analysis, two aliquots of sample PI-02-39 were neutron-irradiated, in order that the extended Ar-Ar technique (21) could be used to determine K and Cl in the same extractions as N and Ar. The combined analyses of Cl, K, Ar, and N isotopes confirm previous studies (9, 13–16) showing that trapped fluids are mixtures of a low-salinity, low-N, and low-radiogenic ^{40}Ar end member, with several hydrothermal components rich in N, Cl, K, and radiogenic ^{40}Ar .

For all samples, step-crushing data define well-resolved mixing correlations in a $^{40}\text{Ar}/^{36}\text{Ar}$ versus $\text{N}_2/^{36}\text{Ar}$ diagram that are consistent with mixing between several hydrothermal fluids and a low- $^{40}\text{Ar}/^{36}\text{Ar}$, $\text{N}_2/^{36}\text{Ar}$ end member (Fig. 1). The slopes of the correlations differ widely between samples, indicating variable enrichment of ^{40}Ar in the different trapped hydrothermal end

members. The lowest measured $\text{N}_2/^{36}\text{Ar}$ ratios in step-crushing runs of PI-02-39 aliquots range from $0.72 \pm 0.03 \times 10^4$ (the last crushing step of PI-02-39-3, table S1 and fig. S3) to $1.43 \pm 0.02 \times 10^4$ (the first crushing step of PI-02-39-4). A higher value of $3.13 \pm 0.02 \times 10^4$ was obtained for aliquot PI-02-39-2; however, we suspect the contribution of hydrothermal N in this case (20). We consider a $\text{N}_2/^{36}\text{Ar}$ range of 0.7×10^4 to 1.4×10^4 as representative of the low-salinity end member of sample PI-02-39. Sample PI-06 presents much lower $^{40}\text{Ar}/^{36}\text{Ar}$ ratios, in the range from 386 to 1014, than those of sample PI-02-39, in agreement with the less evolved character of its trapped fluids (15, 20). Despite this difference, its lowest $\text{N}_2/^{36}\text{Ar}$ ratios (0.85×10^4 to 1.50×10^4 , table S3) are in the same range as those of sample PI-02.

The convergence of data points toward a common end member (Fig. 1) constrains the possible range of $\text{N}_2/^{36}\text{Ar}$ ratios for the presumed Archean ASW value, which is consistent with the modern ASW value. Based on the results summarized in table S3, we propose that the Archean ASW N_2/Ar ratio was $\leq 0.7 \times 10^4$ to 1.4×10^4 , comparable to that of modern ASW (1.0×10^4 to 1.3×10^4). Assuming a constant concentration of atmospheric ^{36}Ar since 3.5 billion years ago (Ga) (6) implies that the Archean

P_{N_2} was $1 \leq 1$ bar (scaled to the modern P_{N_2} of 0.79 bar), and possibly as low as 0.5 bar.

Variations of the N isotopic composition (expressed as $\delta^{15}\text{N}$ relative to the modern atmospheric value) are also consistent with mixing between a crustal/sedimentary end member ($\delta^{15}\text{N}$ within 3 to 8 per mil (‰) for metagabbros (4) and 5 to 15‰ for sediments (4, 14, 22, 23), and an Archean atmospheric $\delta^{15}\text{N}$ value within ~ 2 to 3‰ of the present-day value (Fig. 2). A modern-like N isotope ratio in the Archean is in agreement with the conclusions of a near-constant atmospheric $\delta^{15}\text{N}$ through time from the analysis of ancient cherts (14, 23), although others (24) have proposed drastic ^{15}N enrichments of atmospheric N_2 during the Archean eon. The near-constancy of the atmospheric $\delta^{15}\text{N}$ value and of P_{N_2} since 3.0 to 3.5 Ga is consistent with the presence of a significant terrestrial magnetic field in the Archean. In the absence of such magnetic shielding, atmospheric dinitrogen would have interacted with charged particles from the solar wind, resulting in the nonthermal loss to space of this element and N isotope fractionation (3), as in the atmospheres of Mars (25) and Titan (26). In order to efficiently shield the atmosphere against N_2 loss, a magnetic field at least 50% of the present-day intensity would have been required 3.5 Ga (3), which is in agreement with paleomagnetic data from 3.2 Gy-old single silicate crystals (27).

An Archean atmospheric $P_{\text{N}_2} \leq 1.1$ bar, together with a N isotopic composition similar to that of the present-day atmosphere, has important implications for the thermal conditions of Earth's surface. The energy delivered by the ancient Sun might have been as little as 70% of what it is today, requiring other sources of energy or, more probably, a larger greenhouse effect than today (28). The presence of greenhouse gases such as NH_3 and CH_4 has been proposed [(29) and references therein], but their stability in the ancient atmosphere has been questioned. Others have postulated the occurrence of higher P_{CO_2} in the past, although the geological record of Archean sedimentary rocks suggests that the P_{CO_2} could have been only a few times larger the present-day value (29). Two studies (1, 2) have proposed that a P_{N_2} two to three times the present-day one [with the presence of other gaseous species such as H_2 (2)] was sufficient to maintain a clement surface temperature, with partial pressures of greenhouse gases consistent with the geological record. The present results are not consistent with these proposals and can be used to set an upper limit of the maximum Archean P_{CO_2} pressure. A recent study (30) based on fossil imprints of rain droplets proposed an atmospheric pressure < 2 bar at 2.7 Ga, probably less than an upper limit in the range from 0.5 to 1.14 bar, which is similar to our estimate of the P_{N_2} in the Archean atmosphere (0.5 to 1.1 bar). Although the ages of our Archean samples may differ, this comparison suggests by the difference between the two estimates that the P_{CO_2} was not more than ~ 0.7 bar and may have

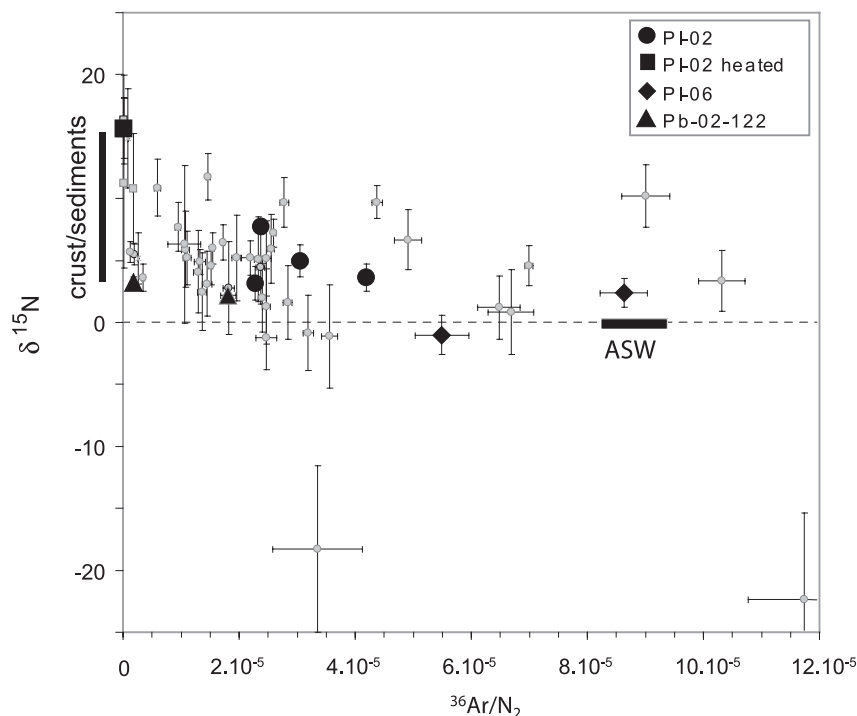


Fig. 2. N isotopic composition ($\delta^{15}\text{N}$ is the deviation in parts per mil from the modern atmospheric $^{15}\text{N}/^{14}\text{N}$ ratio of 3.6765×10^{-3}) versus the $^{36}\text{Ar}/\text{N}_2$ ratio for all extraction steps (small gray symbols) and for the total extracted gases of each sample (large black symbols). In this format, mixings will yield straight lines. All extractions were done by crushing except for a heating run for PI-02-39 (gray and black squares). ASW is the modern air-saturated water composition ($^{36}\text{Ar}/\text{N}_2$ computed with data given in table S3, $\delta^{15}\text{N} = 0\text{‰}$). The range of crustal and sedimentary values is also indicated (4, 14, 22, 23). Observed ratios in Archean fluid inclusions are consistent with mixing between typical crustal/sedimentary values from the hydrothermal end members and an ASW component having a N isotopic composition comparable to the modern one within ~ 2 to 3‰.

been far less. This is qualitatively consistent with conditions necessary to maintain a temperature of $\sim 15^{\circ}\text{C}$ at Earth's surface with a mixture of CO_2 and other greenhouse gases (28, 29).

References and Notes

1. C. Goldblatt *et al.*, *Nat. Geosci.* **2**, 891–896 (2009).
2. R. Wordsworth, R. Pierrehumbert, *Science* **339**, 64–67 (2013).
3. H. I. M. Lichtenegger *et al.*, *Icarus* **210**, 1–7 (2010).
4. V. Busigny, P. Cartigny, P. Philippot, *Geochim. Cosmochim. Acta* **75**, 7502–7521 (2011).
5. R. O. Pepin, *Earth Planet. Sci. Lett.* **252**, 1–14 (2006).
6. The excellent match between the atmospheric $^{38}\text{Ar}/^{36}\text{Ar}$ ratio (two Ar isotopes of primordial origin) and that of Ar trapped in primitive meteorites attests that atmospheric Ar was not quantitatively lost to space [noble gas isotopic ratios are sensitive to thermal and nonthermal escape processes (5)]. Independently, noble gas isotope systematics indicate that mantle degassing did not contribute quantitatively to the atmospheric inventory of these elements after Earth's formation. The large difference in the $^{40}\text{Ar}/^{36}\text{Ar}$ values between the mantle and the atmosphere implies that less than 2% of mantle ^{36}Ar has been transferred to the atmosphere since the time of Earth's formation (31). A similar conclusion is obtained from ^{36}Ar flux estimates. The modern flux of ^{36}Ar from the mantle is ~ 2000 mol/year [from scaling to the present-day ^3He flux from the mantle (32)]. Integrated over 3.5 Gy (the age of the formations studied here) and assuming a constant flux through time, the amount of ^{36}Ar is only about 0.1% of the ^{36}Ar atmospheric inventory (5.55×10^{15} mol). The degassing rate of the mantle was probably higher in the past because of a higher thermal regime. Coltice *et al.* (33) estimated that the melting rate of the mantle was a factor of ~ 20 higher 3.5 Gy ago as compared to the present-day rate.
7. R. Buick, J. S. R. Dunlop, *Sedimentology* **37**, 247–277 (1990).
8. M. J. Van Kranendonk, P. Philippot, K. Lepot, S. Bodorkos, F. Pirajno, *Precambrian Res.* **167**, 93–124 (2008).
9. J. Foriel *et al.*, *Earth Planet. Sci. Lett.* **228**, 451–463 (2004).
10. P. Philippot *et al.*, *C. R. Palevol* **8**, 649–663 (2009).
11. N. Thébaud, P. Philippot, P. Rey, J. Cauzid, *Contrib. Mineral. Petrol.* **152**, 485–503 (2006).
12. N. Thébaud *et al.*, *Earth Planet. Sci. Lett.* **272**, 639–655 (2008).
13. M. Nishizawa, Y. Sano, Y. Ueno, S. Maruyama, *Earth Planet. Sci. Lett.* **254**, 332–344 (2007).
14. D. L. Pinti, K. Hashizume, J. I. Matsuda, *Geochim. Cosmochim. Acta* **65**, 2301–2315 (2001).
15. M. Pujol, B. Marty, R. Burgess, *Earth Planet. Sci. Lett.* **308**, 298–306 (2011).
16. M. Pujol, B. Marty, R. Burgess, G. Turner, P. Philippot, *Nature* **498**, 87–90 (2013).
17. P. G. Burnard, R. Hu, G. Turner, X. W. Bi, *Geochim. Cosmochim. Acta* **63**, 1595–1604 (1999).
18. R. C. Hamme, R. S. Emerson, *Deep Sea Res.* **51**, 1517–1528 (2004).
19. F. Robert, M. Chaussidon, *Nature* **443**, 969–972 (2006).
20. Supplementary materials on Science Online.
21. M. A. Kendrick, R. Burgess, R. A. D. Patrick, G. Turner, *Chem. Geol.* **177**, 351–370 (2001).
22. B. Marty, N. Dauphas, *Earth Planet. Sci. Lett.* **206**, 397–410 (2003).
23. Y. Sano, C. T. Pillinger, *Geochim. J.* **24**, 315–325 (1990).
24. Y. F. Jia, R. Kerrich, *Terra Nova* **16**, 102–108 (2004).
25. T. Owen *et al.*, *J. Geophys. Res.* **82**, 4635–4639 (1977).
26. H. B. Niemann *et al.*, *Nature* **438**, 779–784 (2005).
27. J. A. Tarduno, R. D. Cottrell, M. K. Watkeys, D. Bauch, *Nature* **446**, 657–660 (2007).
28. C. Sagan, G. Mullen, *Science* **177**, 52–56 (1972).
29. J. F. Kasting, *Nature* **464**, 687–689 (2010).
30. S. M. Som, D. C. Catling, J. P. Harnmeijer, P. M. Polivka, R. Buick, *Nature* **484**, 359–362 (2012).
31. M. Ozima, F. A. Podosek, *Noble Gas Geochemistry* (Cambridge Univ. Press, Cambridge, 2002), and references therein.
32. B. Marty, P. Allé, in *Noble Gas Geochemistry and Cosmochemistry*, J. Matsuda, Ed. (Terra Scientific, Tokyo, 1994), pp. 191–204.
33. N. Coltice, B. Marty, R. Yokochi, *Chem. Geol.* **266**, 4–9 (2009).

Acknowledgments: This study was supported by Agence Nationale de la Recherche Grant eLife-2 to P.P. and B.M. and by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007–2013 grant agreement no. 267255 to B.M.). P.P. acknowledges the Institut de Physique du Globe de Paris, the Institut des Sciences de l'Univers, and the Geological Survey of Western Australia for supporting the Pilbara Drilling Project. We thank six anonymous reviewers and D. Catling for constructive comments. This is Centre de Recherches Pétrographiques et Géochimiques contribution no. 2248 and Institut de Physique du Globe de Paris contribution no. 3424.

Supplementary Materials

www.sciencemag.org/content/342/6154/101/suppl/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References (34–41)

23 May 2013; accepted 5 September 2013
Published online 19 September 2013;
10.1126/science.1240971

Following Gene Duplication, Paralog Interference Constrains Transcriptional Circuit Evolution

Christopher R. Baker,^{1,2} Victor Hanson-Smith,^{1,2} Alexander D. Johnson^{1,2*}

Most models of gene duplication assume that the ancestral functions of the preduplication gene are independent and can therefore be neatly partitioned between descendant paralogs. However, many gene products, such as transcriptional regulators, are components within cooperative assemblies; here, we show that a natural consequence of duplication and divergence of such proteins can be competitive interference between the paralogs. Our example is based on the duplication of the essential MADS-box transcriptional regulator *Mcm1*, which is found in all fungi and regulates a large set of genes. We show that a set of historical amino acid sequence substitutions minimized paralog interference in contemporary species and, in doing so, increased the molecular complexity of this gene regulatory network. We propose that paralog interference is a common constraint on gene duplicate evolution, and its resolution, which can generate additional regulatory complexity, is needed to stabilize duplicated genes in the genome.

Gene duplications are an important source of new genes, and a variety of models have been developed to rationalize why

certain gene duplicates have been maintained over evolutionary time (1–3). For instance, the neofunctionalization model posits that soon after duplication, one of the duplicates evolves a new function that can be selected for and, thereby, maintained over time (2, 3). Alternatively, subfunctionalization (via the duplication-degeneration-complementation model) holds that duplicates can be maintained in the genome by acquiring

reciprocal loss-of-function mutations, such that both duplicates become necessary to perform the combined functions of the preduplication ancestor (1–3). Classically, these models have assumed that ancestral functions can be treated independently, making the partitioning of these functions among the descendant paralogs possible without detrimental effects (2). However, for the many gene products that participate in cooperative assemblies, the molecular interactions that underlie gene functions are not intrinsically independent (4). For example, many transcriptional regulators depend on a cooperative network of protein-protein and protein-nucleic acid interactions. In these instances, loss of one or more ancestral molecular interactions will often give rise to competitive interference between gene duplicates (paralog interference) (5). Although in some instances this competition may be advantageous, we suspect that paralog interference following gene duplication would typically have detrimental effects that must be evolutionarily bypassed for the paralogs to be maintained. Because many proteins form cooperative assemblies, resolution of paralog interference is likely to be a widespread phenomenon influencing the fate of duplicated genes.

Mcm1 is a fungal MADS-box transcriptional regulator that binds DNA cooperatively with seven different partner transcriptional regulators (cofactors) to control the expression of many genes, including those coding for mating functions and arginine metabolic enzymes (6). The way in which

¹Department of Immunology and Microbiology, University of California, San Francisco, CA 94143, USA. ²Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA.

*Corresponding author. E-mail: ajohnson@cgl.ucsf.edu



Following Gene Duplication, Paralog Interference Constrains Transcriptional Circuit Evolution

Christopher R. Baker *et al.*

Science **342**, 104 (2013);

DOI: 10.1126/science.1240810

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/104.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.104.DC1.html>

This article **cites 37 articles**, 20 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/104.full.html#ref-list-1>

been far less. This is qualitatively consistent with conditions necessary to maintain a temperature of $\sim 15^{\circ}\text{C}$ at Earth's surface with a mixture of CO_2 and other greenhouse gases (28, 29).

References and Notes

1. C. Goldblatt *et al.*, *Nat. Geosci.* **2**, 891–896 (2009).
2. R. Wordsworth, R. Pierrehumbert, *Science* **339**, 64–67 (2013).
3. H. I. M. Lichtenegger *et al.*, *Icarus* **210**, 1–7 (2010).
4. V. Busigny, P. Cartigny, P. Philippot, *Geochim. Cosmochim. Acta* **75**, 7502–7521 (2011).
5. R. O. Pepin, *Earth Planet. Sci. Lett.* **252**, 1–14 (2006).
6. The excellent match between the atmospheric $^{38}\text{Ar}/^{36}\text{Ar}$ ratio (two Ar isotopes of primordial origin) and that of Ar trapped in primitive meteorites attests that atmospheric Ar was not quantitatively lost to space [noble gas isotopic ratios are sensitive to thermal and nonthermal escape processes (5)]. Independently, noble gas isotope systematics indicate that mantle degassing did not contribute quantitatively to the atmospheric inventory of these elements after Earth's formation. The large difference in the $^{40}\text{Ar}/^{36}\text{Ar}$ values between the mantle and the atmosphere implies that less than 2% of mantle ^{36}Ar has been transferred to the atmosphere since the time of Earth's formation (31). A similar conclusion is obtained from ^{36}Ar flux estimates. The modern flux of ^{36}Ar from the mantle is ~ 2000 mol/year [from scaling to the present-day ^3He flux from the mantle (32)]. Integrated over 3.5 Gy (the age of the formations studied here) and assuming a constant flux through time, the amount of ^{36}Ar is only about 0.1% of the ^{36}Ar atmospheric inventory (5.55×10^{15} mol). The degassing rate of the mantle was probably higher in the past because of a higher thermal regime. Coltice *et al.* (33) estimated that the melting rate of the mantle was a factor of ~ 20 higher 3.5 Gy ago as compared to the present-day rate.
7. R. Buick, J. S. R. Dunlop, *Sedimentology* **37**, 247–277 (1990).
8. M. J. Van Kranendonk, P. Philippot, K. Lepot, S. Bodorkos, F. Pirajno, *Precambrian Res.* **167**, 93–124 (2008).
9. J. Foriel *et al.*, *Earth Planet. Sci. Lett.* **228**, 451–463 (2004).
10. P. Philippot *et al.*, *C. R. Palevol* **8**, 649–663 (2009).
11. N. Thébaud, P. Philippot, P. Rey, J. Cauzid, *Contrib. Mineral. Petrol.* **152**, 485–503 (2006).
12. N. Thébaud *et al.*, *Earth Planet. Sci. Lett.* **272**, 639–655 (2008).
13. M. Nishizawa, Y. Sano, Y. Ueno, S. Maruyama, *Earth Planet. Sci. Lett.* **254**, 332–344 (2007).
14. D. L. Pinti, K. Hashizume, J. I. Matsuda, *Geochim. Cosmochim. Acta* **65**, 2301–2315 (2001).
15. M. Pujol, B. Marty, R. Burgess, *Earth Planet. Sci. Lett.* **308**, 298–306 (2011).
16. M. Pujol, B. Marty, R. Burgess, G. Turner, P. Philippot, *Nature* **498**, 87–90 (2013).
17. P. G. Burnard, R. Hu, G. Turner, X. W. Bi, *Geochim. Cosmochim. Acta* **63**, 1595–1604 (1999).
18. R. C. Hamme, R. S. Emerson, *Deep Sea Res.* **51**, 1517–1528 (2004).
19. F. Robert, M. Chaussidon, *Nature* **443**, 969–972 (2006).
20. Supplementary materials on Science Online.
21. M. A. Kendrick, R. Burgess, R. A. D. Patrick, G. Turner, *Chem. Geol.* **177**, 351–370 (2001).
22. B. Marty, N. Dauphas, *Earth Planet. Sci. Lett.* **206**, 397–410 (2003).
23. Y. Sano, C. T. Pillinger, *Geochim. J.* **24**, 315–325 (1990).
24. Y. F. Jia, R. Kerrich, *Terra Nova* **16**, 102–108 (2004).
25. T. Owen *et al.*, *J. Geophys. Res.* **82**, 4635–4639 (1977).
26. H. B. Niemann *et al.*, *Nature* **438**, 779–784 (2005).
27. J. A. Tarduno, R. D. Cottrell, M. K. Watkeys, D. Bauch, *Nature* **446**, 657–660 (2007).
28. C. Sagan, G. Mullen, *Science* **177**, 52–56 (1972).
29. J. F. Kasting, *Nature* **464**, 687–689 (2010).
30. S. M. Som, D. C. Catling, J. P. Harnmeijer, P. M. Polivka, R. Buick, *Nature* **484**, 359–362 (2012).
31. M. Ozima, F. A. Podosek, *Noble Gas Geochemistry* (Cambridge Univ. Press, Cambridge, 2002), and references therein.
32. B. Marty, P. Allé, in *Noble Gas Geochemistry and Cosmochemistry*, J. Matsuda, Ed. (Terra Scientific, Tokyo, 1994), pp. 191–204.
33. N. Coltice, B. Marty, R. Yokochi, *Chem. Geol.* **266**, 4–9 (2009).

Acknowledgments: This study was supported by Agence Nationale de la Recherche Grant eLife-2 to P.P. and B.M. and by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007–2013 grant agreement no. 267255 to B.M.). P.P. acknowledges the Institut de Physique du Globe de Paris, the Institut des Sciences de l'Univers, and the Geological Survey of Western Australia for supporting the Pilbara Drilling Project. We thank six anonymous reviewers and D. Catling for constructive comments. This is Centre de Recherches Pétrographiques et Géochimiques contribution no. 2248 and Institut de Physique du Globe de Paris contribution no. 3424.

Supplementary Materials

www.sciencemag.org/content/342/6154/101/suppl/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References (34–41)

23 May 2013; accepted 5 September 2013
Published online 19 September 2013;
10.1126/science.1240971

Following Gene Duplication, Paralog Interference Constrains Transcriptional Circuit Evolution

Christopher R. Baker,^{1,2} Victor Hanson-Smith,^{1,2} Alexander D. Johnson^{1,2*}

Most models of gene duplication assume that the ancestral functions of the preduplication gene are independent and can therefore be neatly partitioned between descendant paralogs. However, many gene products, such as transcriptional regulators, are components within cooperative assemblies; here, we show that a natural consequence of duplication and divergence of such proteins can be competitive interference between the paralogs. Our example is based on the duplication of the essential MADS-box transcriptional regulator *Mcm1*, which is found in all fungi and regulates a large set of genes. We show that a set of historical amino acid sequence substitutions minimized paralog interference in contemporary species and, in doing so, increased the molecular complexity of this gene regulatory network. We propose that paralog interference is a common constraint on gene duplicate evolution, and its resolution, which can generate additional regulatory complexity, is needed to stabilize duplicated genes in the genome.

Gene duplications are an important source of new genes, and a variety of models have been developed to rationalize why

certain gene duplicates have been maintained over evolutionary time (1–3). For instance, the neofunctionalization model posits that soon after duplication, one of the duplicates evolves a new function that can be selected for and, thereby, maintained over time (2, 3). Alternatively, subfunctionalization (via the duplication-degeneration-complementation model) holds that duplicates can be maintained in the genome by acquiring

reciprocal loss-of-function mutations, such that both duplicates become necessary to perform the combined functions of the preduplication ancestor (1–3). Classically, these models have assumed that ancestral functions can be treated independently, making the partitioning of these functions among the descendant paralogs possible without detrimental effects (2). However, for the many gene products that participate in cooperative assemblies, the molecular interactions that underlie gene functions are not intrinsically independent (4). For example, many transcriptional regulators depend on a cooperative network of protein-protein and protein-nucleic acid interactions. In these instances, loss of one or more ancestral molecular interactions will often give rise to competitive interference between gene duplicates (paralog interference) (5). Although in some instances this competition may be advantageous, we suspect that paralog interference following gene duplication would typically have detrimental effects that must be evolutionarily bypassed for the paralogs to be maintained. Because many proteins form cooperative assemblies, resolution of paralog interference is likely to be a widespread phenomenon influencing the fate of duplicated genes.

Mcm1 is a fungal MADS-box transcriptional regulator that binds DNA cooperatively with seven different partner transcriptional regulators (cofactors) to control the expression of many genes, including those coding for mating functions and arginine metabolic enzymes (6). The way in which

¹Department of Immunology and Microbiology, University of California, San Francisco, CA 94143, USA. ²Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA.

*Corresponding author. E-mail: ajohnson@cgl.ucsf.edu

Mcm1 assembles at the arginine metabolism (*ARG*) genes varies between fungal clades. In the yeasts *Kluyveromyces lactis* and *Candida albicans*, an Mcm1 homodimer regulates transcription of *ARG* genes by binding specifically to DNA with the cofactor Arg81 (Fig. 1A) (7, 8). In the lineage leading to baker's yeast (*Saccharomyces cerevisiae*), a tandem gene duplication event introduced an extra copy of Mcm1 (called Arg80), such that the

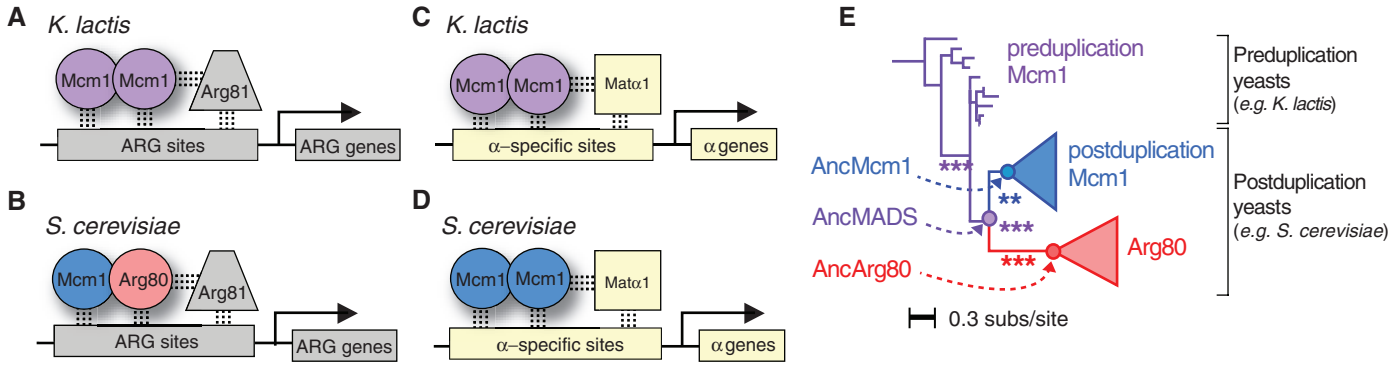


Fig. 1. Function and evolution of MADS-box proteins in hemiascomycete yeasts. (A) In *K. lactis*, an Mcm1 homodimer regulates the *ARG* genes by interacting with Arg81 and binding a specific DNA sequence. (B) In *S. cerevisiae*, an Mcm1-Arg80 heterodimer interacts with Arg81 to regulate *ARG* genes. (C) An Mcm1 homodimer interacts with Matα1 to regulate α-specific genes in *K. lactis* and (D) *S. cerevisiae*. (E) A maximum likelihood phylogeny of MADS-box do-

main proteins in hemiascomycete yeasts. A tandem gene duplication generated paralogs Mcm1 and Arg80 in the last shared common ancestor of *Zygosaccharomyces rouxii* and *S. cerevisiae*. Circles denote ancestral proteins reconstructed in this study. Asterisks on internal branches correspond to approximate-likelihood ratio support for the monophyly of the descendant clade: *** denotes support > 10.0; ** denotes support > 5.0. subs, substitutions.

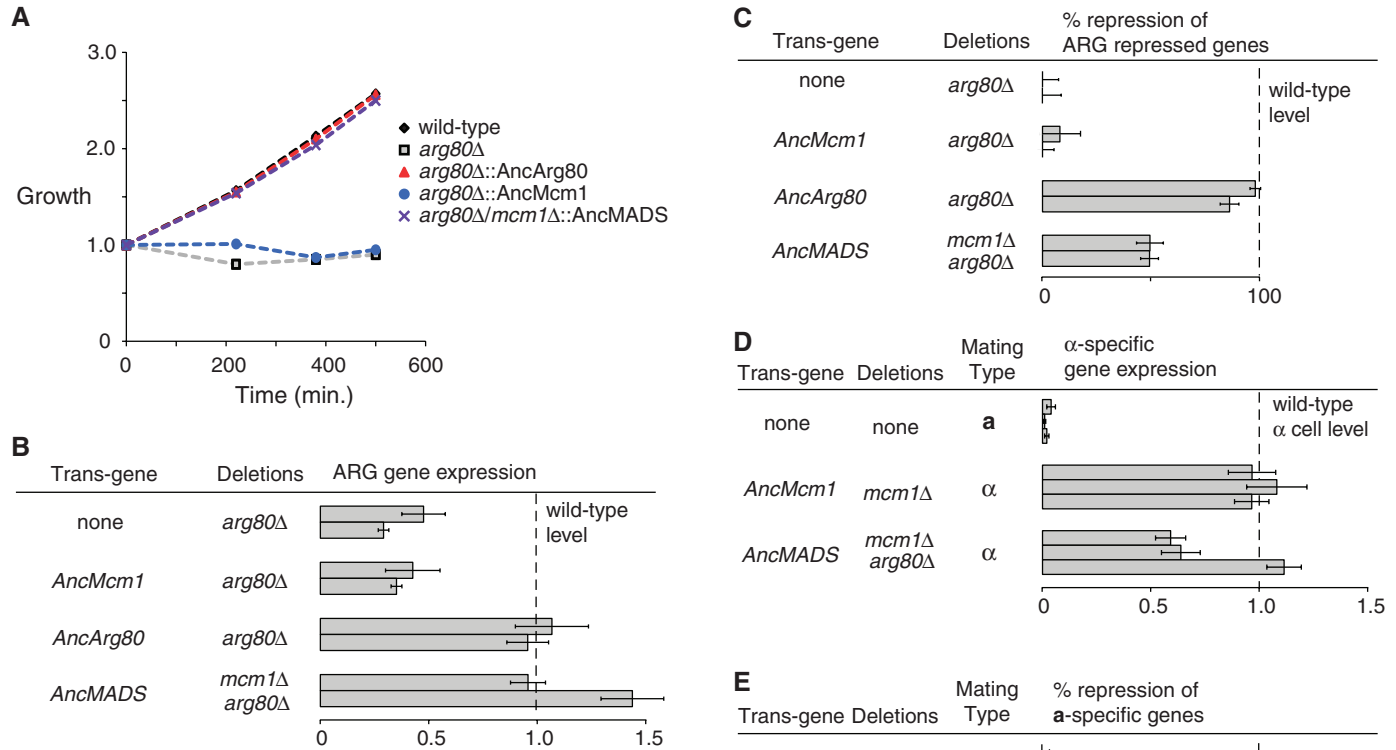


Fig. 2. The preduplication ancestral gene complements both paralogs. (A) Growth of ancestral MADS-box gene strains using ornithine as a sole nitrogen source. Ornithine is converted into arginine and then modified to produce the other essential amino acids. In the absence of a functional *ARG* gene regulatory complex, strains cannot use ornithine as a nitrogen source. The preduplication AncMADS can supply the function of the modern Arg80 paralog (purple), but the postduplication AncMcm1 paralog cannot (blue). "Growth" on the y axis is the ratio of optical density at 600 nm (OD₆₀₀) at the indicated time point divided by OD₆₀₀ at time zero. (B to E) Gene expression profiling of ancestral MADS-box proteins in *S. cerevisiae* quantified with NanoString (www.nanostring.com). (B) MADS-box activated *ARG* genes. Row 1, *CAR1*; row 2, *CAR2*. (C) MADS-box repressed *ARG* genes. Row 1,

ARG3; row 2, *ARG5,6*. (D) MADS-box activated mating genes (α-specific genes). Row 1, *SAG1*; row 2, *Mfa1*; row 3, *STE3*. (E) MADS-box repressed mating genes (α-specific genes). Row 1, *STE2*; row 2, *STE6*. In each experiment, mean and standard error (indicated by error bars) were determined using three replicates.

S. cerevisiae regulatory architecture is more complex. In *S. cerevisiae*, an Mcm1-Arg80 heterodimer regulates the transcription of *ARG* genes by binding DNA with the cofactor Arg81 (Fig. 1B) (9). Other Mcm1-regulated gene sets in *S. cerevisiae* did not experience an increase in regulatory complexity following gene duplication. For instance, the α -specific genes (genes that give α mating cells their specialized properties) are regulated by an Mcm1 homodimer that binds specifically to DNA with the cofactor Mata1 in species that branch before and after the gene duplication event (Fig. 1, C and D) (10–12). In all instances, gene regulation by Mcm1 and Arg80 depends on the formation of strong interactions with both cofactors and DNA.

To understand how the linked biochemical functions of DNA and cofactor binding diverged after Mcm1 duplicated, we reconstructed ancestral MADS-box proteins, characterized these ancestral proteins in vivo and in vitro, and identified the mutations through which their functions diversified [see supplementary materials and methods and (13)]. Specifically, we reconstructed the MADS-box domains of the most recent common shared ancestor of all postduplication Mcm1 paralogs (AncMcm1); all postduplication Arg80 paralogs (AncArg80); and the preduplication, most recent shared common ancestor of all Mcm1 and Arg80 paralogs (AncMADS) (Fig. 1E, fig. S1, and tables S1 and S2).

We integrated the reconstructed ancestral MADS-box proteins into *S. cerevisiae* and re-

moved the modern copies of *ARG80* and *MCMI* to determine if the ancestral proteins could complement their deletion. Deletion of *S. cerevisiae MCMI* is lethal, and deletion of *S. cerevisiae ARG80* produces defects in arginine metabolism (14, 15). We found that the preduplication AncMADS protein complemented both defects: Replacement of Mcm1 or Arg80 with AncMADS had no impact on growth in either rich media or media with a precursor of arginine as a sole nitrogen source, a phenotype that depends on normal *ARG* gene regulation (Fig. 2A and fig. S2A). We measured the expression levels of a representative set of Mcm1 and Arg80/Mcm1 regulated genes and found that AncMADS restored activation and repression of these genes, and most genes showed the same dynamic range as in the wild type (Fig. 2, B to E, and fig. S2B). The exceptions were a diminished dynamic range of gene expression for the *ARG* repressed genes, a mildly diminished dynamic range of gene expression for the α -specific genes, and stronger activation than the wild type for the *ARG* activated gene *CAR2* (Fig. 2, B to D). In contrast, the postduplication MADS-box proteins (AncArg80 and AncMcm1) failed to complement deletions of the sister paralogs. Specifically, AncMcm1 did not complement the deletion of the native *ARG80* and, similarly, the presence of AncArg80 alone did not rescue the deletion of *MCMI* (an essential gene) (Fig. 2, A to C, and fig. S2C). The capacity of the preduplication ancestral MADS-box protein to complement the functions of both daugh-

ter genes in a modern species, combined with the inability of the postduplication ancestors to do the same, shows that AncMcm1 and AncArg80 acquired degenerative mutations that necessitated the retention of both paralogs over evolutionary time.

We next determined the mutations that underlie the diversification of AncMcm1 and AncArg80 following the duplication of AncMADS. The cofactors Mata1 and Arg81 both interact with the same portion of the MADS-box domain, which we refer to as the cofactor binding pocket (16, 17). We modeled the reconstructed protein sequences for AncMADS, AncMcm1, and AncArg80 onto the structure of *S. cerevisiae* Mcm1 in complex with DNA (18) and then compared the sequences within the cofactor binding pocket (fig. S3, A to C). On the lineage from AncMADS to AncMcm1, one substitution occurred in the pocket [Tyr³³→Phe³³ (Y33F); Y, Tyr; F, Phe], and it has been conserved in all Mcm1 descendants (Fig. 3A and table S1). On the lineage from AncMADS to AncArg80, three sequence substitutions occurred within the cofactor binding pocket (T41A, Q42N, F62L; T, Thr; A, Ala; Q, Gln; N, Asn; L, Leu), and each has been strongly conserved in postduplication Arg80 descendant sequences (Fig. 3A and table S1). Previous work has shown that these residues play a critical role in stabilizing the interactions between Arg81 and Arg80, as well as Mata1 and Mcm1 (16, 17). To assess the impact of these changes on the preduplication ancestor, we introduced these mutations into AncMADS and observed

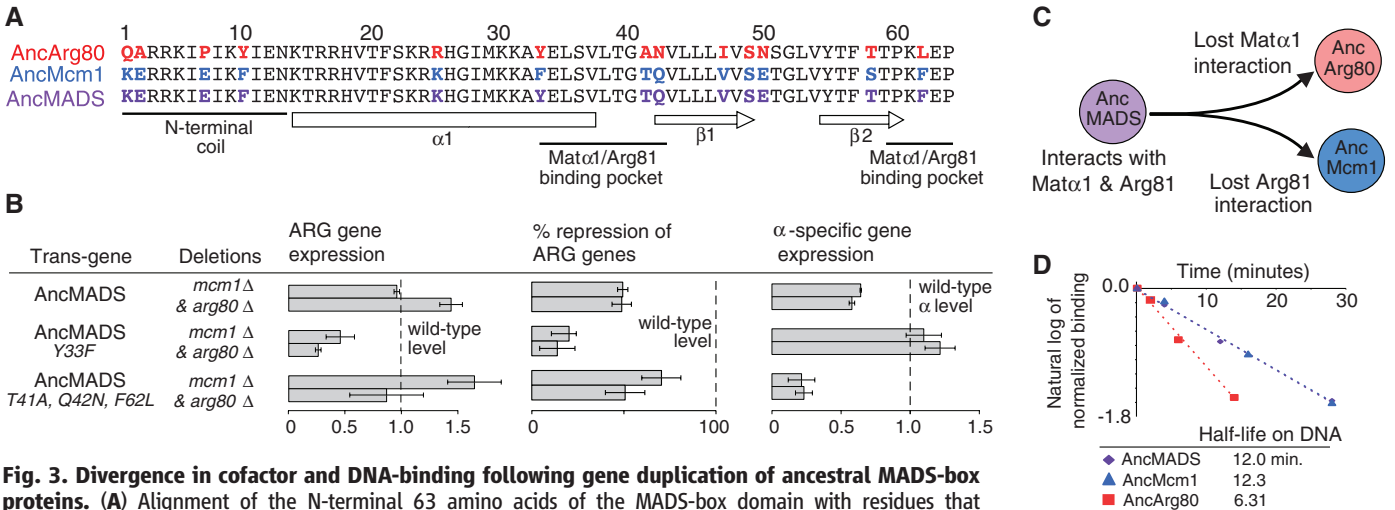


Fig. 3. Divergence in cofactor and DNA-binding following gene duplication of ancestral MADS-box proteins. (A) Alignment of the N-terminal 63 amino acids of the MADS-box domain with residues that changed identity between AncMcm1, AncArg80, and AncMADS in color (24). α 1 denotes a long α helix; β 1 and β 2 signify an antiparallel β sheet. (B) Gene expression profiling to determine the impact of mutants on the function of preduplication AncMADS protein in *S. cerevisiae*. Gene expression quantified using NanoString. Panel 1: MADS-box activated *ARG* genes; row 1, *CAR1*; row 2, *CAR2*. Panel 2: MADS-box repressed *ARG* genes; row 1, *ARG3*; row 2, *ARG5,6*. Panel 3: MADS-box activated mating genes (α -specific genes); row 1, *SAG1*; row 2, *MF α 1*. Mean and standard error (indicated by error bars) were determined from three replicates. (C) After duplication, AncArg80 lost the ability to form a strong interaction with Mata1, and AncMcm1 lost the ability to form a strong interaction with Arg81. These losses destroyed the abilities of AncArg80 and AncMcm1 to regulate α -specific genes and *ARG* genes, respectively. (D) Half-lives of MADS-box ancestors on the *S. cerevisiae CAR2* cis-regulatory sequence (see supplementary materials and methods). Saturating levels of unlabeled DNA were added at time point zero. (E) After the duplication of AncMADS, α -specific genes are regulated by a homodimer of AncMcm1, whereas *ARG* genes are regulated by a heterodimer of AncMcm1 and AncArg80 due to the reduced affinity of AncArg80 for DNA.

their effect on expression levels of *ARG* genes and α -specific genes. When we introduced the Mcm1 substitution (Y33F) into AncMADS, we observed a disruption of *ARG* gene regulation but no decrease in α -specific gene regulation; in fact, the dynamic range of α -specific gene regulation slightly expanded (Fig. 3B). When we introduced the Arg80 substitutions (T41A, Q42N, F62L) into AncMADS, we observed decreased α -specific gene regulation but no compromise in *ARG* gene regulation (Fig. 3B). (We note that *CAR2* even returns to wild-type expression levels from its elevated state in the AncMADS background.) We observed similar effects of these mutations on AncMADS when we swapped in the cofactors Arg81 and Mata1 from the preduplication species *K. lactis*, indicating that changes to these two cofactors did not play a major role in partitioning the ancestral molecular interactions of AncMADS (fig. S3, D and E). Taken together, these results show that the preduplication AncMADS could form cofactor interactions with both Arg81 and Mata1 and that these interactions reciprocally degenerated in the descendant paralogs: AncMcm1 lost its ability to productively interact with Arg81, whereas AncArg80 lost its ability to interact with Mata1 (Fig. 3C).

We next examined the DNA-binding surfaces of the pre- and postduplication MADS-box proteins. *S. cerevisiae* Arg80 and Mcm1 have very closely related DNA-binding specificities (19, 20), indicating that, at most, a limited divergence in MADS-box DNA-binding specificity occurred following duplication. That the DNA-binding specificity did not change substantially is also supported by our observation that the preduplication AncMADS protein can complement the deletion of either postduplication gene (Fig. 2). However, *S. cerevisiae* Arg80 has a substantially lower affinity for DNA than Mcm1 (15, 16). To determine when this affinity change occurred, we compared the DNA-binding affinities of AncMADS, AncArg80, and AncMcm1 by measuring their half-lives on DNA. To make the comparisons meaningful, we used an endogenous *S. cerevisiae* MADS-box binding site (taken from an arginine metabolic gene) that closely resembles the consensus site for *S. cerevisiae* Mcm1 and Arg80 and that has been

shown to support binding by both these proteins in vitro (8, 20). We observed that the half-life of AncArg80 was significantly lower than the half-lives of AncMADS and AncMcm1 (Fig. 3D). Thus, the difference in affinity between modern Mcm1 and Arg80 is due to a decrease in Arg80 DNA-binding affinity that occurred soon after the duplication (Fig. 3E).

Next, we investigated the consequences of this reduction in Arg80 DNA-binding affinity on gene expression. We hypothesized that a version of Arg80 with full DNA-binding strength might interfere with Mcm1 by binding to α -specific gene regulatory sites and acting as a dominant negative mutant by preventing Mcm1 from binding cooperatively with Mata1 (Fig. 4A). If this were true, then the reduction of Arg80 DNA-binding affinity would minimize competition by weighting DNA-binding at the α -specific genes in favor of Mcm1. To test this idea, we increased the DNA-binding affinity of AncArg80 to that of the preduplication protein and measured the extent of competitive interference with Mcm1 in *S. cerevisiae*. We identified a total of five mutations (K1Q, E2A, E7P, F10Y, K25R; K, Lys; E, Glu; P, Pro; R, Arg) that occurred on the DNA-binding surface of AncArg80 after the AncMADS duplication (Fig. 3A). A subset of these residues are known to affect MADS-box DNA-binding affinity in *S. cerevisiae* (14, 16). We reversed these mutations in AncArg80, returning the DNA-binding region of AncArg80 to its preduplication, high-affinity state, and then measured α -specific gene expression in an *S. cerevisiae* strain lacking the native Arg80. As predicted by our hypothesis (paralog interference), the AncArg80 mutant significantly reduced α -specific gene expression (Fig. 4B). When overexpressed, the nonmutant, low-affinity AncArg80 protein dampened α -specific gene expression, and the overexpressed, high-affinity AncArg80 mutant blocked the expression of the α -specific genes almost entirely (Fig. 4B). [These effects were not an indirect consequence of altering *MCM1* gene expression levels (fig. S4A).] The antagonism between Arg80 and Mcm1 persists in an attenuated form in contemporary species, as the deletion of *ARG80* in *S. cerevisiae* slightly increases α -specific gene expression (Fig.

4B and fig. S4B). On the basis of these observations, we conclude that the historical reduction in Arg80 DNA-binding affinity limited the degree of paralog interference between Arg80 and Mcm1 at the α -specific genes.

The diminished DNA-binding affinity of Arg80 also provides a simple explanation for the origins of the Arg80-Mcm1 heterodimer (as opposed to an Arg80 homodimer) at the *ARG* genes in *S. cerevisiae*. The cofactor Arg81 contacts only a single (proximal) subunit within the MADS-box dimer, and this interaction will favor Arg80 because of its strong interaction with Arg81. The energetics will favor Mcm1, rather than Arg80, as the second (distal) subunit because of its higher affinity for DNA (Fig. 3E).

By combining ancestral gene reconstructions with the biochemical and genetic tools available for yeasts, we have shown that competition between paralogs arose as an intrinsic consequence of the duplication and subfunctionalization of a deeply conserved transcriptional regulator. This interference was minimized by a set of historical amino acid substitutions, and we suggest that this was necessary for both paralogs to be maintained, as without the weakened affinity of Arg80 for DNA, gene regulation is severely compromised (Fig. 4B). The minimization of interference was accompanied by an increase in regulatory complexity: The increased number of distinct subunits needed to regulate the *ARG* genes in *S. cerevisiae* relative to the preduplication ancestor (three versus two) is necessary to compensate for the reduced DNA-binding affinity of Arg80. Although we do not know whether the mutations that affected protein-protein interactions occurred before, after, or in concert with those that affected DNA-binding, we have shown that each mutation is a loss-of-function (or at least a reduction-of-function) amino acid substitution (21, 22).

For the many gene products that form cooperative assemblies, exemplified by the proteins studied here, ancestral functions depend on a network of molecular interactions. Following gene duplication, the loss of ancestral interactions by such proteins, resulting in subfunctionalization, may unavoidably give rise to dominant negative

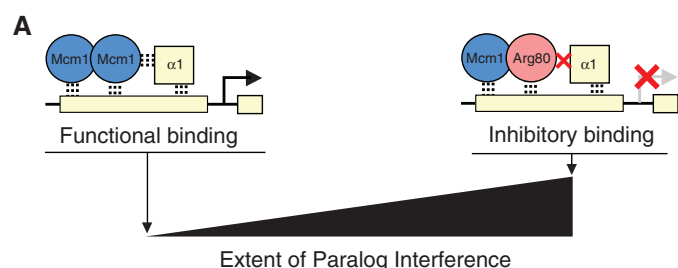


Fig. 4. Paralog interference between Arg80 and Mcm1. (A) The ratio of functional binding to inhibitory binding determines the extent of competitive interference between Mcm1 and Arg80. (B) Arg80, AncArg80, and AncArg80 mutant with DNA-binding surface changes reverted to ancestral state interfere with α -specific gene expression. α -specific gene expression was quantified by

quantitative reverse-transcriptase fluorescence polymerase chain reaction using *SAG1* transcript (normalized to *URA6* transcript). Endogenous expression is driven by the native *ARG80* promoter, and overexpression is driven by the *TEF1* promoter. For gene expression experiments, mean and standard error (indicated by error bars) were determined from five replicates.

effects between duplicates. Although in some cases, this interference can be exploited, for example, by using it to repress gene expression (5, 23), we propose that a more common outcome is the minimization of this interference in gene duplicates that persist over evolutionary time. Whether such minimization is generally accompanied by an increase in regulatory complexity, as seen here, remains to be determined.

References and Notes

1. A. Force *et al.*, *Genetics* **151**, 1531–1545 (1999).
2. H. Innan, F. Kondrashov, *Nat. Rev. Genet.* **11**, 97–108 (2010).
3. A. Wagner, *Genome Biol.* **3**, reviews1012.1–reviews1012.3 (2002).
4. A. Wagner, *Proc. Biol. Sci.* **270**, 457–466 (2003).
5. J. T. Bridgham, J. E. Brown, A. Rodríguez-Marí, J. M. Catchen, J. W. Thornton, *PLOS Genet.* **4**, e1000191 (2008).
6. F. Messenguy, E. Dubois, *Gene* **316**, 1–21 (2003).
7. C. Boonchird, F. Messenguy, E. Dubois, *Mol. Gen. Genet.* **226**, 154–166 (1991).
8. B. B. Tuch, D. J. Galgoczy, A. D. Hernday, H. Li, A. D. Johnson, *PLOS Biol.* **6**, e38 (2008).
9. F. Messenguy, E. Dubois, *Mol. Cell. Biol.* **13**, 2586–2592 (1993).
10. A. Bender, G. F. Sprague Jr., *Cell* **50**, 681–691 (1978).
11. A. E. Tsong, M. G. Miller, R. M. Raisner, A. D. Johnson, *Cell* **115**, 389–399 (2003).
12. C. R. Baker, B. B. Tuch, A. D. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7493–7498 (2011).
13. M. J. Harms, J. W. Thornton, *Curr. Opin. Struct. Biol.* **20**, 360–366 (2010).
14. T. B. Acton, J. Mead, A. M. Steiner, A. K. Vershon, *Mol. Cell. Biol.* **20**, 1–11 (2000).
15. N. Amar, F. Messenguy, M. El Bakkoury, E. Dubois, *Mol. Cell. Biol.* **20**, 2087–2097 (2000).
16. A. Jamaï, E. Dubois, A. K. Vershon, F. Messenguy, *Mol. Cell. Biol.* **22**, 5741–5752 (2002).
17. J. Mead *et al.*, *Mol. Cell. Biol.* **22**, 4607–4621 (2002).
18. S. Tan, T. J. Richmond, *Nature* **391**, 660–666 (1998).
19. T. E. Hayes, P. Sengupta, B. H. Cochran, *Genes Dev.* **2**, 1713–1722 (1988).
20. F. Messenguy, E. Dubois, C. Boonchird, *Mol. Cell. Biol.* **11**, 2852–2863 (1991).
21. G. C. Finnigan, V. Hanson-Smith, T. H. Stevens, J. W. Thornton, *Nature* **481**, 360–364 (2012).
22. M. Lynch, J. S. Conery, *Science* **302**, 1401–1404 (2003).
23. M. Y. Dennis *et al.*, *Cell* **149**, 912–922 (2012).
24. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Acknowledgments: We thank B. Tuch for assistance and comments; O. Homann for assistance on experimental techniques; and C. Nobile, C. Dalal, S. Devika, R. Bennett, L. Mera, and T. Sorrells for comments. This work was funded by grant RO1 GM057049 from the NIH.

Supplementary Materials

www.sciencemag.org/content/342/6154/104/suppl/DC1

Materials and Methods

Figs. S1 to S4

Tables S1 to S5

References (25–38)

21 May 2013; accepted 6 September 2013

10.1126/science.1240810

Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs

Jasper M. de Goeij,^{1*} Dick van Oevelen,² Mark J. A. Vermeij,³ Ronald Osinga,⁴ Jack J. Middelburg,⁵ Anton F. P. M. de Goeij,⁶ Wim Admiraal¹

Ever since Darwin's early descriptions of coral reefs, scientists have debated how one of the world's most productive and diverse ecosystems can thrive in the marine equivalent of a desert. It is an enigma how the flux of dissolved organic matter (DOM), the largest resource produced on reefs, is transferred to higher trophic levels. Here we show that sponges make DOM available to fauna by rapidly expelling filter cells as detritus that is subsequently consumed by reef fauna. This "sponge loop" was confirmed in aquarium and in situ food web experiments, using ¹³C- and ¹⁵N-enriched DOM. The DOM-sponge-fauna pathway explains why biological hot spots such as coral reefs persist in oligotrophic seas—the reef's paradox—and has implications for reef ecosystem functioning and conservation strategies.

Coral reefs thrive in oligotrophic tropical seas, but nevertheless belong to the most productive ecosystems on Earth (1–3). Efficient retention and recycling of carbon and nutrients causes the net production of reefs to

be close to zero, despite high gross primary production (4). Reef primary producers such as corals and algae release up to 50% of their fixed carbon (5, 6), of which up to 80% immediately dissolves in seawater (7). This shunt into the dissolved organic matter (DOM) pool represents a major flow of energy and nutrients on coral reefs (7). In the open ocean, microbes enable the transfer of DOM to higher trophic levels through the well-established microbial loop (8). Studies on coral reefs have therefore also initially focused on microbes in reef waters and adjacent permeable sediments to understand the fate of DOM in these systems (7, 9–11). However, uptake rates by bacterioplankton, in the sense of the microbial loop, are largely insufficient to explain the observed DOM removal on Caribbean and Indo-Pacific reefs (12). It therefore remains unclear how the largest source of

energy and nutrients on reefs is transferred to higher trophic levels.

Cryptic habitats, for example, the coral reef's crevices and cavities, are identified as major sinks of DOM on Caribbean and Indo-Pacific reefs (12). These habitats cover up to two-thirds of the reef's volume, and the biomass of cryptic organisms can exceed that on the open reef (13, 14). DOM removal rates in cryptic habitats on Caribbean reefs (12) are comparable to the average gross primary production rates of the entire coral reef ecosystem (2). DOM removal rates on Indo-Pacific reefs are lower (12) but still account for up to 46% of the average gross reef productivity. Sponges are primarily responsible for total DOM uptake and remove the same amount of DOM from the water column in 30 min as free-living bacteria take up in 30 days (12, 15). Therefore, sponges retain organic matter within the reef community and thereby prevent energy and nutrient losses to the open ocean. Surprisingly however, sponges respire only 42% of the carbon taken up from the surrounding water (15, 16). Assuming that the remaining 58% is used for growth, a biomass increase of 38% of body carbon per day (more than a doubling of biomass every 3 days) would be expected (16). In reality, however, the net growth rate of sponges is near zero (15, 16), implying high losses of sponge biomass through a rapid tissue turnover.

A rapid turnover and extensive loss of sponge cells to the surrounding water has been shown for the sponge *Halisarca caerulea* (17). The sponge's filter cells (choanocytes) divide every 5 to 6 hours, representing the fastest cell cycle found in any multicellular organism to date (17). This rapid cell production is counterbalanced by massive shedding of old choanocytes as particulate organic matter (POM or detritus) into the water column (17). Massive shedding of POM is also observed in other tropical sponges (18, 19).

¹Department of Aquatic Ecology and Ecotoxicology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Post Office Box 94248, 1090 GE Amsterdam, Netherlands.

²Department of Ecosystem Studies, NIOZ Royal Netherlands Institute for Sea Research, NL-4400 AC Yerseke, Netherlands.

³Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Carmabi, Piscaderabaai z/n Willemstad, Curaçao. ⁴Department of Aquaculture and Fisheries, Wageningen University, Post Office Box 338, 6700 AH Wageningen, Netherlands. ⁵Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, Netherlands. ⁶Faculty of Health, Medicine and Life Sciences, Maastricht University, Post Office Box 616, 6200 MD Maastricht, Netherlands.

*Corresponding author. E-mail: j.m.degoeij@uva.nl



Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs

Jasper M. de Goeij *et al.*

Science **342**, 108 (2013);

DOI: 10.1126/science.1241981

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/108.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/03/342.6154.108.DC1.html>

This article **cites 36 articles**, 3 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/108.full.html#ref-list-1>

effects between duplicates. Although in some cases, this interference can be exploited, for example, by using it to repress gene expression (5, 23), we propose that a more common outcome is the minimization of this interference in gene duplicates that persist over evolutionary time. Whether such minimization is generally accompanied by an increase in regulatory complexity, as seen here, remains to be determined.

References and Notes

1. A. Force *et al.*, *Genetics* **151**, 1531–1545 (1999).
2. H. Innan, F. Kondrashov, *Nat. Rev. Genet.* **11**, 97–108 (2010).
3. A. Wagner, *Genome Biol.* **3**, reviews1012.1–reviews1012.3 (2002).
4. A. Wagner, *Proc. Biol. Sci.* **270**, 457–466 (2003).
5. J. T. Bridgham, J. E. Brown, A. Rodríguez-Marí, J. M. Catchen, J. W. Thornton, *PLOS Genet.* **4**, e1000191 (2008).
6. F. Messenguy, E. Dubois, *Gene* **316**, 1–21 (2003).
7. C. Boonchird, F. Messenguy, E. Dubois, *Mol. Gen. Genet.* **226**, 154–166 (1991).
8. B. B. Tuch, D. J. Galgoczy, A. D. Hernday, H. Li, A. D. Johnson, *PLOS Biol.* **6**, e38 (2008).
9. F. Messenguy, E. Dubois, *Mol. Cell. Biol.* **13**, 2586–2592 (1993).
10. A. Bender, G. F. Sprague Jr., *Cell* **50**, 681–691 (1978).
11. A. E. Tsong, M. G. Miller, R. M. Raisner, A. D. Johnson, *Cell* **115**, 389–399 (2003).
12. C. R. Baker, B. B. Tuch, A. D. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 7493–7498 (2011).
13. M. J. Harms, J. W. Thornton, *Curr. Opin. Struct. Biol.* **20**, 360–366 (2010).
14. T. B. Acton, J. Mead, A. M. Steiner, A. K. Vershon, *Mol. Cell. Biol.* **20**, 1–11 (2000).
15. N. Amar, F. Messenguy, M. El Bakkoury, E. Dubois, *Mol. Cell. Biol.* **20**, 2087–2097 (2000).
16. A. Jamaï, E. Dubois, A. K. Vershon, F. Messenguy, *Mol. Cell. Biol.* **22**, 5741–5752 (2002).
17. J. Mead *et al.*, *Mol. Cell. Biol.* **22**, 4607–4621 (2002).
18. S. Tan, T. J. Richmond, *Nature* **391**, 660–666 (1998).
19. T. E. Hayes, P. Sengupta, B. H. Cochran, *Genes Dev.* **2**, 1713–1722 (1988).
20. F. Messenguy, E. Dubois, C. Boonchird, *Mol. Cell. Biol.* **11**, 2852–2863 (1991).
21. G. C. Finnigan, V. Hanson-Smith, T. H. Stevens, J. W. Thornton, *Nature* **481**, 360–364 (2012).
22. M. Lynch, J. S. Conery, *Science* **302**, 1401–1404 (2003).
23. M. Y. Dennis *et al.*, *Cell* **149**, 912–922 (2012).
24. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Acknowledgments: We thank B. Tuch for assistance and comments; O. Homann for assistance on experimental techniques; and C. Nobile, C. Dalal, S. Devika, R. Bennett, L. Mera, and T. Sorrells for comments. This work was funded by grant RO1 GM057049 from the NIH.

Supplementary Materials

www.sciencemag.org/content/342/6154/104/suppl/DC1

Materials and Methods

Figs. S1 to S4

Tables S1 to S5

References (25–38)

21 May 2013; accepted 6 September 2013

10.1126/science.1240810

Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs

Jasper M. de Goeij,^{1*} Dick van Oevelen,² Mark J. A. Vermeij,³ Ronald Osinga,⁴ Jack J. Middelburg,⁵ Anton F. P. M. de Goeij,⁶ Wim Admiraal¹

Ever since Darwin's early descriptions of coral reefs, scientists have debated how one of the world's most productive and diverse ecosystems can thrive in the marine equivalent of a desert. It is an enigma how the flux of dissolved organic matter (DOM), the largest resource produced on reefs, is transferred to higher trophic levels. Here we show that sponges make DOM available to fauna by rapidly expelling filter cells as detritus that is subsequently consumed by reef fauna. This "sponge loop" was confirmed in aquarium and in situ food web experiments, using ¹³C- and ¹⁵N-enriched DOM. The DOM-sponge-fauna pathway explains why biological hot spots such as coral reefs persist in oligotrophic seas—the reef's paradox—and has implications for reef ecosystem functioning and conservation strategies.

Coral reefs thrive in oligotrophic tropical seas, but nevertheless belong to the most productive ecosystems on Earth (1–3). Efficient retention and recycling of carbon and nutrients causes the net production of reefs to

be close to zero, despite high gross primary production (4). Reef primary producers such as corals and algae release up to 50% of their fixed carbon (5, 6), of which up to 80% immediately dissolves in seawater (7). This shunt into the dissolved organic matter (DOM) pool represents a major flow of energy and nutrients on coral reefs (7). In the open ocean, microbes enable the transfer of DOM to higher trophic levels through the well-established microbial loop (8). Studies on coral reefs have therefore also initially focused on microbes in reef waters and adjacent permeable sediments to understand the fate of DOM in these systems (7, 9–11). However, uptake rates by bacterioplankton, in the sense of the microbial loop, are largely insufficient to explain the observed DOM removal on Caribbean and Indo-Pacific reefs (12). It therefore remains unclear how the largest source of

energy and nutrients on reefs is transferred to higher trophic levels.

Cryptic habitats, for example, the coral reef's crevices and cavities, are identified as major sinks of DOM on Caribbean and Indo-Pacific reefs (12). These habitats cover up to two-thirds of the reef's volume, and the biomass of cryptic organisms can exceed that on the open reef (13, 14). DOM removal rates in cryptic habitats on Caribbean reefs (12) are comparable to the average gross primary production rates of the entire coral reef ecosystem (2). DOM removal rates on Indo-Pacific reefs are lower (12) but still account for up to 46% of the average gross reef productivity. Sponges are primarily responsible for total DOM uptake and remove the same amount of DOM from the water column in 30 min as free-living bacteria take up in 30 days (12, 15). Therefore, sponges retain organic matter within the reef community and thereby prevent energy and nutrient losses to the open ocean. Surprisingly however, sponges respire only 42% of the carbon taken up from the surrounding water (15, 16). Assuming that the remaining 58% is used for growth, a biomass increase of 38% of body carbon per day (more than a doubling of biomass every 3 days) would be expected (16). In reality, however, the net growth rate of sponges is near zero (15, 16), implying high losses of sponge biomass through a rapid tissue turnover.

A rapid turnover and extensive loss of sponge cells to the surrounding water has been shown for the sponge *Halisarca caerulea* (17). The sponge's filter cells (choanocytes) divide every 5 to 6 hours, representing the fastest cell cycle found in any multicellular organism to date (17). This rapid cell production is counterbalanced by massive shedding of old choanocytes as particulate organic matter (POM or detritus) into the water column (17). Massive shedding of POM is also observed in other tropical sponges (18, 19).

¹Department of Aquatic Ecology and Ecotoxicology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Post Office Box 94248, 1090 GE Amsterdam, Netherlands.

²Department of Ecosystem Studies, NIOZ Royal Netherlands Institute for Sea Research, NL-4400 AC Yerseke, Netherlands.

³Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Carmabi, Piscaderabaai z/n Willemstad, Curaçao. ⁴Department of Aquaculture and Fisheries, Wageningen University, Post Office Box 338, 6700 AH Wageningen, Netherlands. ⁵Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, Netherlands. ⁶Faculty of Health, Medicine and Life Sciences, Maastricht University, Post Office Box 616, 6200 MD Maastricht, Netherlands.

*Corresponding author. E-mail: j.m.degoeij@uva.nl

This suggests that sponges use the majority of incorporated carbon to rejuvenate their filter system and maintain a high cell turnover.

We hypothesize here that shed sponge cells (detritus) are subsequently ingested by particle-feeding organisms (detritivores). Sponges thereby make the energy and nutrients stored in the DOM pool available to organisms at higher trophic levels that would otherwise be unable to capitalize on this resource. Because small detritivores (such as crustaceans and polychaetes) are themselves fed upon by larger animals higher in the food

web, sponges are at the base of a sponge loop that ultimately recycles energy and nutrients back into the ecosystem in a similar way as the microbial loop does in the open ocean.

To study the proposed DOM-sponge-detritus feedback loop on coral reefs, we tested three key predictions of this hypothesis: (i) sponges take up DOM, (ii) sponges convert DOM into detritus, and (iii) sponge-derived detritus is taken up by detritivores. These three predictions were first tested in flow chambers in a controlled running-seawater aquarium setup (fig. S1) using ^{13}C - and

^{15}N -enriched DOM, extracted from the cosmopolitan marine diatom *Phaeodactylum tricornutum*, as a food web tracer (20).

All three key elements were confirmed experimentally (Fig. 1). Four common reef sponge species showed uptake of dissolved organic carbon (DO^{13}C) and nitrogen (DO^{15}N) (Fig. 1A). All four species subsequently produced ^{13}C - and ^{15}N -enriched detritus (Fig. 1B). The four sponge species converted 11 to 24% of the assimilated DO^{13}C into detritus (PO^{13}C) and 18 to 36% of the DO^{15}N into PO^{15}N within 3 hours (Fig. 1, A and B). Control incubations showed that detritus production without sponges was less than 4% of the detritus production in incubations with sponges. Detritivores subsequently fed on the labeled sponge-derived detritus (Fig. 1C). Isotopically enriched detritus, collected from specimens of the four tested sponge species (fig. S2) that were repeatedly fed with ^{13}C - and ^{15}N -enriched DOM (20), was added to six cores containing cavity sediments with residing fauna and, in three out of six cores, motile fauna were added (hermit crabs and snails) (fig. S1). Within 6 hours, sponge detritus was incorporated by 17 out of 28 (^{13}C) and 23 out of 28 (^{15}N) specimens of detritivores.

After experimental confirmation of a sponge loop in flow chambers, the question arose whether this newly found pathway could actually be identified in a complex coral reef environment. Therefore, the water exchange of two in situ cryptic reef cavities (75 and 100 liters) with the surrounding reef water was temporarily restricted (12, 20), and ^{13}C - and ^{15}N -enriched DOM was injected into the enclosed cavity at the start of two consecutive incubation periods of 3 hours (fig. S3). Once we restored the water exchange between the water column and the cavities, the presence and fate of labeled DOM were analyzed over the subsequent 45 hours within the main cavity compartments; that is, sponges, sponge-derived detritus, surface sediment, bacterioplankton, nonsponge filter feeders, and motile fauna such as hermit crabs and snails (20). The relative abundance of ^{13}C and ^{15}N in these compartments over time provided qualitative

Fig. 1. Fate of DOM tracer ^{13}C (red bars) and ^{15}N (blue bars) through sponge-driven DOM transfer in flow chamber experiments.

(A) Uptake of tracer DOM (DO^{13}C and DO^{15}N); micromoles of tracer per millimole of sponge C or N $\pm$ SD; $n = 4$ specimens by the sponge species *Halisarca caerulea* (Hc), *Haliclona implexiformis* (Hi), *Chondrilla caribensis* (Cc), and *Scopalina ruetzleri* (Sr). The uptake of DO^{13}C by sponges is further specified in tissue assimilation (dark red bars) and respiration (light red bars). (B) Production of detritus (PO^{13}C and PO^{15}N); micromoles of tracer per millimole of sponge C or N $\pm$ SD; $n = 4$ specimens by the sponges HC, HI, CC, and SR. (C) Uptake of sponge-derived tracer detritus (PO^{13}C and PO^{15}N); micromoles of tracer per millimole of faunal C or N $\pm$ SD by detritivores ($n = 28$ specimens) picked from six cores of reef sediment; three cores were supplemented with hermit crabs and snails.

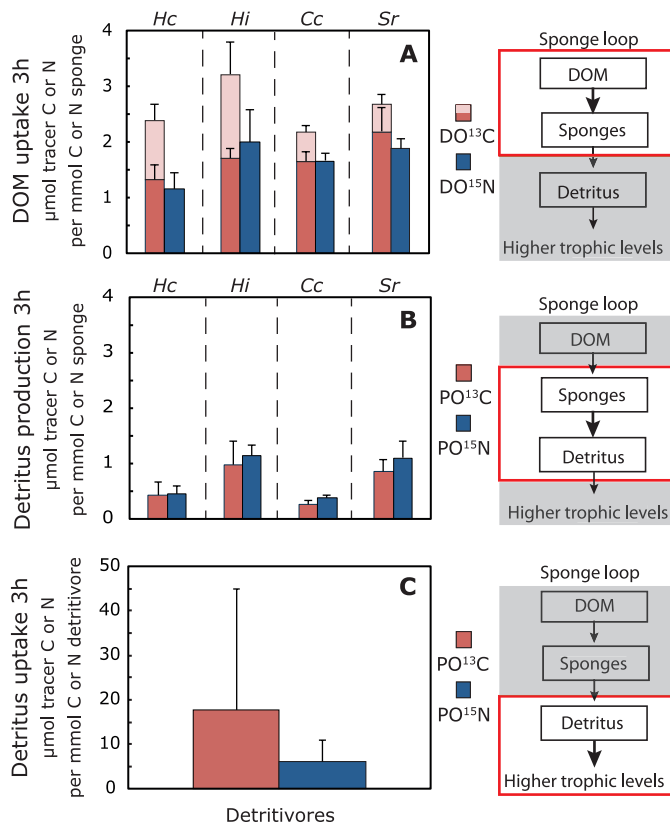
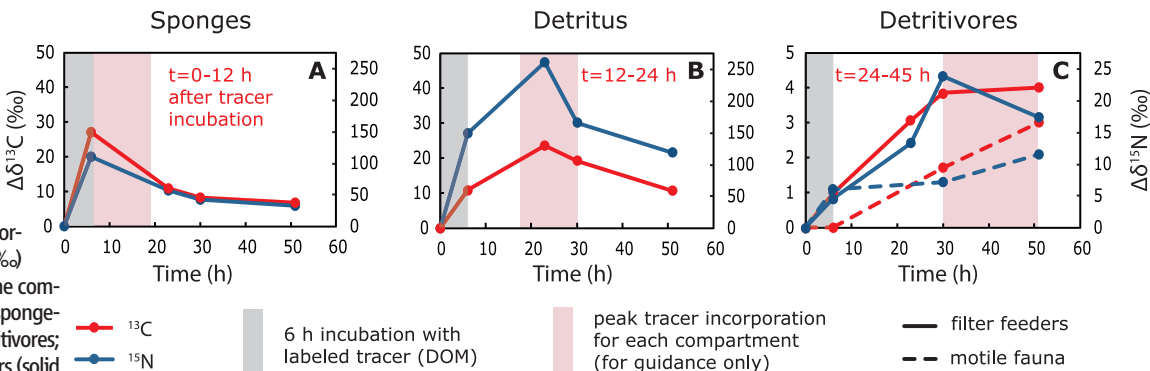


Fig. 2. In situ sponge-driven transfer of tracer DOM (red line, ^{13}C ; blue line, ^{15}N) in coral reef cavities after a temporary 6-hour closure (gray shading) and the subsequent 45 hours.

The mean above-background isotope tracer incorporation ($\Delta\delta^{13}\text{C}\%$ and $\Delta\delta^{15}\text{N}\%$) of two cavities is shown for the compartments (A) sponges, (B) sponge-derived detritus, and (C) detritivores; that is, nonsponge filter feeders (solid line) and motile fauna (dashed line). For guidance, the interval of peak tracer incorporation is highlighted for each compartment. t , time; h, hours.



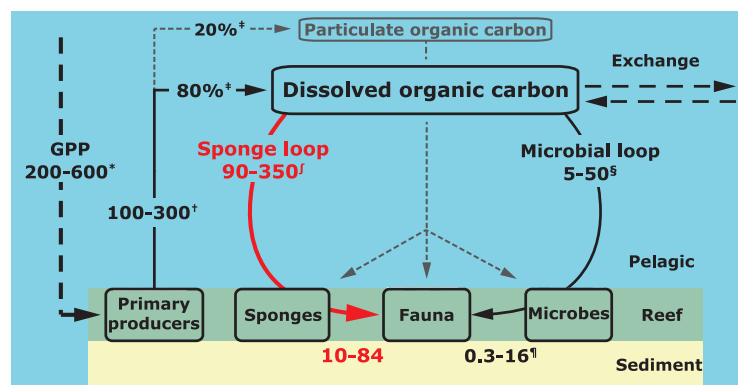


Fig. 3. A simplified scheme of dominant pathways (millimoles of $C\ m^{-2}\ day^{-1}$) of organic carbon transfer on coral reefs in the pelagic (blue), benthic reef (green), and sediment (yellow) ecosystem compartments. The proposed sponge loop (red arrow) is shown in addition to the classical microbial loop. GPP, gross primary production. * (2), † (5, 6), ‡ (7), § (12, 11, 12) ¶ (11); see (20) for details.

evidence in support of our proposed pathway of sponge-driven DOM transfer (Fig. 2). After the introduction of labeled DOM to coral cavities, the uptake of tracer ^{13}C and ^{15}N was first observed in sponges (first prediction: DOM-sponge; mean sponge $\delta^{13}C$ 27 per mil (‰) and $\delta^{15}N$ 111‰; Fig. 2A) immediately after the 6-hour incubation period. Between 12 and 24 hours after the incubation, the relative isotope abundance peaked in sponge-derived detritus (second prediction: sponge-detritus; mean detritus $\delta^{13}C$ 24‰ and $\delta^{15}N$ 261‰; Fig. 2B). The sponge-derived detritus was finally transferred into motile fauna and nonsponge filter feeders after 45 hours (third prediction: detritus-higher trophic levels; steady increase to a detritivore $\delta^{13}C$ 3 to 4‰ and $\delta^{15}N$ 12 to 17‰; Fig. 2C). The $^{13}C:^{15}N$ ratio of sponge-derived detritus was lower than the $^{13}C:^{15}N$ of the sponges (Fig. 2, A and B), indicating that the detritus was relatively enriched in N. The DOM-derived $\delta^{13}C$ or $\delta^{15}N$ in the surface sediment or the bacterioplankton was generally lower than 2‰, indicating limited uptake by these compartments.

The seemingly paradoxical observation that productive ecosystems such as coral reefs thrive in nutrient-poor waters can only be explained through processes ensuring efficient capture, retention, and recycling of energy and nutrients. Such tight recycling mechanisms involve microbial processing of coral- and algal-derived DOM in the water column and permeable reef sands (7, 11). Here we show that, in addition to the transfer of DOM via bacteria to fauna (8), sponges transform the majority of DOM into particulate detritus, a pathway that has hitherto not been recognized (Fig. 3). The underlying mechanisms of DOM uptake and rapid cell turnover in sponges are not yet fully understood. Sponges form close associations with microorganisms, forming so-called holobionts, and both sponge cells and microbes can assimilate DOM (16), although their relative contributions remain largely unknown. The sponge loop nevertheless greatly enhances our growing understanding of the efficiency that

typifies coral reefs, thus supporting reef life, increasing biodiversity (21, 22), and maintaining high productivity. Sponges not only recycle the energy retained in DOM but also provide reef fauna with a source of nutrients (such as N), thereby fertilizing the coral reef ecosystem. The efficient and fast uptake, retention, and release (23) of nutrients within the originally oligotrophic ecosystem by sponges may also catalyze nutrient-induced shifts in the coral-algal-microbe community after eutrophication, often associated with coral reef degradation (24, 25). Top-down-controlled shifts from coral- to sponge-dominated reefs have been predicted (26) and recorded in the Caribbean (27, 28), but still sponges are rarely considered in analyses of alternative stable states on coral reefs. Other oligotrophic ecosystems where sponges are abundant, such as deep-sea cold-water coral reefs and temperate Mediterranean reefs, may also sustain the functioning of a sponge loop. Deep-sea sponges contribute substantially to the respiration of cold-water reef communities (29) and produce large amounts of detritus (30). Mediterranean reefs are dominated by (cryptic) sponges, of which several abundant species are found to take up DOM (31). Although this study shows the presence of the sponge loop mainly qualitatively, DOM turnover by sponges (15) approaches the daily gross primary production of the entire reef ecosystem (2, 4), suggesting that this energetic pathway is of great ecological importance (Fig. 3). Recognition of the key role of sponges in coral reefs has, consequently, implications for studies on ecosystem services and conservation strategies in ecosystems where sponges are a ubiquitous component.

References and Notes

- H. T. Odum, E. P. Odum, *Ecol. Monogr.* **25**, 291–320 (1955).
- B. G. Hatcher, *Trends Ecol. Evol.* **5**, 149–155 (1990).
- M. J. Atkinson, J. L. Falter, in *Biogeochemistry of Marine Systems*, K. D. Black, G. B. Shimmield, Eds. (Blackwell Publishing, Oxford, 2003), pp. 40–64.
- C. Crossland, B. Hatcher, S. Smith, *Coral Reefs* **10**, 55–64 (1991).

- Y. Tanaka, T. Miyajima, I. Koike, T. Hayashibara, H. Ogawa, *Bull. Mar. Sci.* **82**, 237–245 (2008).
- A. F. Haas et al., *J. Exp. Mar. Biol. Ecol.* **389**, 53–60 (2010).
- C. Wild et al., *Nature* **428**, 66–70 (2004).
- F. Azam et al., *Mar. Ecol. Prog. Ser.* **10**, 257–263 (1983).
- Z. Dubinsky, I. Berman-Frank, *Aquat. Sci.* **63**, 4–17 (2001).
- C. E. Nelson, A. L. Alldredge, E. A. McCliment, L. A. Amaral-Zettler, C. A. Carlson, *ISME J.* **5**, 1374–1387 (2001).
- A. F. Haas et al., *PLOS ONE* **6**, e27973 (2011).
- J. M. de Goeij, F. C. van Duyl, *Limnol. Oceanogr.* **52**, 2608–2617 (2007).
- C. Richter, M. Wunsch, M. Rasheed, I. Kötter, M. I. Badran, *Nature* **413**, 726–730 (2001).
- S. Scheffers, G. Nieuwland, R. Bak, F. van Duyl, *Coral Reefs* **23**, 413–422 (2004).
- J. M. de Goeij, H. van den Berg, M. M. van Oostveen, E. H. G. Epping, F. C. van Duyl, *Mar. Ecol. Prog. Ser.* **357**, 139–151 (2008).
- J. M. de Goeij, L. Moodley, M. Houtekamer, N. M. Carballeira, F. C. van Duyl, *Limnol. Oceanogr.* **53**, 1376–1386 (2008).
- J. M. de Goeij et al., *J. Exp. Biol.* **212**, 3892–3900 (2009).
- H. M. Reyswig, *Biol. Bull.* **141**, 568–591 (1971).
- G. Yahel, J. Sharp, D. Marie, C. Hase, A. Genin, *Limnol. Oceanogr.* **48**, 141–149 (2003).
- Materials and methods are available as supplementary materials on Science Online.
- M. Slattery, D. J. Gochfield, C. G. Easson, L. R. K. O'Donahue, *Mar. Ecol. Prog. Ser.* **476**, 71–86 (2013).
- B. W. Bowen, L. A. Rocha, R. J. Toonen, S. A. Karl; the ToBo Laboratory, *Trends Ecol. Evol.* **28**, 359–366 (2013).
- M. W. Southwell, J. B. Weisz, C. S. Martens, N. Lindquist, *Limnol. Oceanogr.* **53**, 986–996 (2008).
- T. P. Hughes et al., *Curr. Biol.* **17**, 360–365 (2007).
- S. A. Sandin et al., *PLoS One* **3**, e1548 (2008).
- M. González-Rivero, L. Yakob, P. J. Mumby, *Ecol. Modell.* **222**, 1847–1853 (2011).
- A. V. Norström, M. Nystrom, J. Lokrantz, C. Folke, *Mar. Ecol. Prog. Ser.* **376**, 295–306 (2009).
- S. E. McMurray, T. P. Henkel, J. R. Pawlik, *Ecology* **91**, 560–570 (2010).
- D. van Oevelen et al., *Limnol. Oceanogr.* **54**, 1829–1844 (2009).
- U. Witte, T. Brattegard, G. Graf, B. Springer, *Mar. Ecol. Prog. Ser.* **154**, 241–252 (1997).
- M. Ribes et al., *Environ. Microbiol.* **14**, 1224–1239 (2012).

Acknowledgments: J.M.d.G. and D.v.O. contributed equally to this work. We thank D. M. de Bakker, C. G. W. Whalen, and B. E. Alexander for their technical assistance in the field and lab; C. E. Mueller, and P. van Rijswijk for preparation of the DOM substrate, lab assistance, and isotope analysis; and E. R. Hunting for discussion. This work was supported financially by the Innovational Research Incentives Scheme of the Netherlands Organization for Scientific Research (NWO-VENI; 863.10.009, granted to J.M.d.G.) and the Schure-Beijerinck-Popping fund. J.M.d.G. and R.O. are also affiliated with Porifarma BV Poelbos 3, 6718 HT Ede, Netherlands. The authors declare no conflicts of interest. A detailed description of all materials and methods and additional data are available as supplementary materials. All authors discussed the experimental data and jointly wrote the manuscript. The data are deposited in DRYAD, accession no. <http://doi.org/10.5061/dryad.4gb17>.

Supplementary Materials

www.sciencemag.org/content/342/6154/108/suppl/DC1
Materials and Methods
Figs. S1 to S3
References (32–39)

17 June 2013; accepted 6 September 2013
10.1126/science.1241981



Allele-Specific Silencing of Mutant *Myh6* Transcripts in Mice Suppresses Hypertrophic Cardiomyopathy

Jianming Jiang *et al.*

Science **342**, 111 (2013);

DOI: 10.1126/science.1236921

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/111.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.111.DC1.html>

This article **cites 26 articles**, 8 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/111.full.html#ref-list-1>

Allele-Specific Silencing of Mutant *Myh6* Transcripts in Mice Suppresses Hypertrophic Cardiomyopathy

Jianming Jiang,^{1,3,4*} Hiroko Wakimoto,^{1,2,3*} J. G. Seidman,^{1†} Christine E. Seidman^{1,3,4†‡}

Dominant mutations in sarcomere proteins such as the myosin heavy chains (MHC) are the leading genetic causes of human hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy. We found that expression of the HCM-causing cardiac MHC gene (*Myh6*) R403Q mutation in mice can be selectively silenced by an RNA interference (RNAi) cassette delivered by an adeno-associated virus vector. RNAi-transduced MHC^{403/+} mice developed neither hypertrophy nor myocardial fibrosis, the pathologic manifestations of HCM, for at least 6 months. Because inhibition of HCM was achieved by only a 25% reduction in the levels of the mutant transcripts, we suggest that the variable clinical phenotype in HCM patients reflects allele-specific expression and that partial silencing of mutant transcripts may have therapeutic benefit.

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterized by an increase in left ventricular wall thickness (LVWT), disorganization of cardiomyocytes, and expansion of myocardial fibrosis that occurs in the absence of systemic disease (1–3). HCM is the leading cause of nonviolent sudden death in young adults and the most common cause of sudden death on the athletic field (4). HCM is caused by mutations in genes that encode protein constituents of the cardiac sarcomere, the contractile unit of muscle (5, 6). More than 1000 distinct pathogenic mutations have been identified, and more than half of these occur in *MYH7* (encoding β myosin heavy chain) and *MYBPC3* (encoding cardiac myosin binding protein C) (7). Most HCM mutations, and all that occur in *MYH7*, are missense mutations, producing amino acid substitutions in myosin that perturb the sarcomere's contractile function.

The human HCM-causing mutation *MYH7* R403Q (Arg⁴⁰³ → Gln) causes particularly severe disease that is characterized by early-onset and progressive myocardial dysfunction, with a high incidence of sudden cardiac death (8). Heterozygous MHC^{403/+} mice express the R403Q mutation in *Myh6* under the control of the endogenous *Myh* locus. *Myh6* and *MYH7* are highly homologous in sequence and encode the predominant myosin isoforms in the adult hearts. MHC^{403/+} mice recapitulate human HCM and develop hypertrophy, myocyte disarray, and increased myocardial fibrosis (9). Analyses of mutant myosins isolated from MHC^{403/+} mice showed that HCM mutations cause fundamental changes in sarco-

mere functions, including increased acto-myosin sliding velocity, force generation, and adenosine triphosphate hydrolysis (10). These changes in turn alter calcium cycling and gene transcription in myocytes and ultimately induce pathologic remodeling of the heart in vivo (11–13). Understanding this pathogenic cascade has led to the identification of secondary signaling molecules as potential therapeutic targets (13, 14), but no strategies have been defined that correct the primary biophysical and biochemical abnormalities of sarcomeres with HCM mutations.

Selective reduction in the expression of the mutant protein would be the most direct approach for correcting sarcomere dysfunction. As a first step in pursuing this strategy, we determined whether in vivo allele-specific repression of *Myh6* R403Q was feasible. Because mice hemizygous for a normal *Myh6* gene are viable, fertile, and have essentially normal cardiac function (15), we reasoned that inactivation of the mutant sarcomere protein allele is unlikely to have adverse cardiovascular effects. We used an RNA interference (RNAi) construct because this powerful tool has successfully reduced gene expression in many systems and can distinguish between genes that differ by a single nucleotide (16). We selected adeno-associated virus packed with serotype 9 capsid (AAV-9) as a delivery vehicle because this vector has strong tropism for cardiac tissues (17, 18). To enhance the cardiac tropism, we engineered the vector so that AAV-9 expression was under the control of the cardiac-specific troponin T (cTnT) promoter.

We produced 17 unique RNAi constructs, each cotransfected with a plasmid carrying the *Myh6* R403Q mutant gene into 293T human embryonic kidney cells (fig. S1A). One RNAi construct, designated 403m, significantly reduced *Myh6* R403Q expression (Fig. 1, A and B). To assess its specificity, we transfected wild-type or mutant *Myh6* into 293T cells with 403m constructs. Because there was significant silencing (~80%) of both wild-type and mutant *Myh6* expression, we introduced an additional mismatch into the

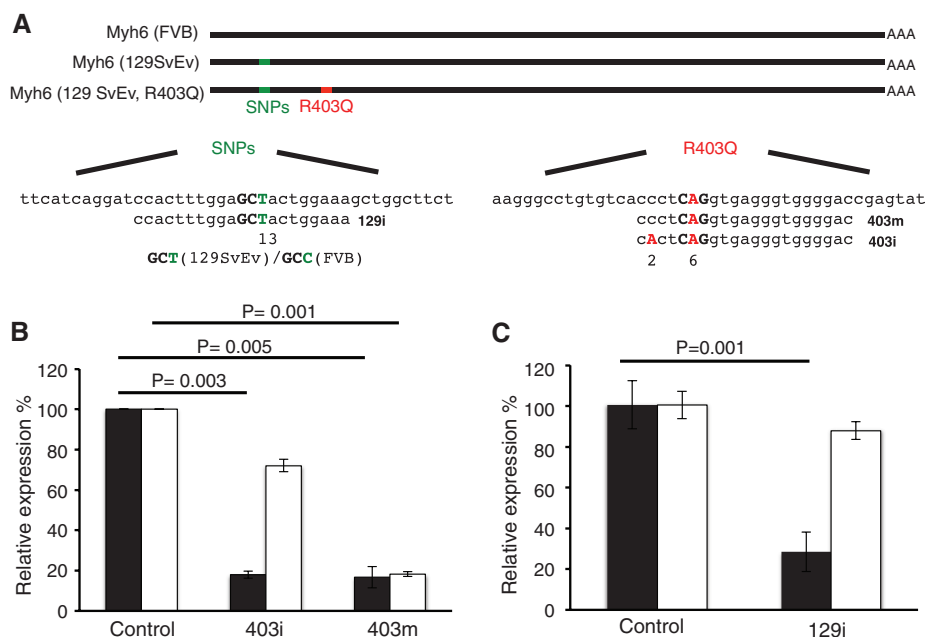


Fig. 1. Selective silencing of *Myh6* R403Q expression by AAV-9-mediated RNAi. (A) Schematic representation of FVB, 129SvEv, and 129SvEv mutant (R403Q) transcript and RNAi sequences. **(B)** Quantitative real-time polymerase chain reaction (PCR) analysis of wild-type *Myh6* (white bar) and mutant *Myh6* R403Q (black bar) expression after transduction of the 403m and 403i constructs ($n = 4$). Levels of the transcripts were normalized to control LacZ RNAi. **(C)** Quantitative real-time PCR analysis of FVB *Myh6* (white bar) and 129SvEv *Myh6* R403Q (black bar) expression after transduction of the 129i construct ($n = 4$). Levels of the transcripts were normalized to control LacZ RNAi. Data are means $\pm$ SD.

¹Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. ²Department of Cardiology, Boston Children's Hospital, Boston, MA 02115, USA. ³Division of Cardiovascular Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA. ⁴Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA.

*These authors contributed equally to this work.

†These authors contributed equally to this work.

‡Corresponding author. E-mail: cseidman@genetics.med.harvard.edu

403m RNAi construct (designated 403i; Fig. 1A). The 403i construct had modest reduction (~20%) of wild-type *Myh6* expression but retained ~80% reduction in the expression of *Myh6* R403Q transcript in 293T cells (Fig. 1B).

To ascertain the cardiac selectivity of AAV-9-cTnT vector, we used enhanced green fluorescent protein (EGFP) (fig. S1B). Virus was injected [5×10^{13} vector genomes (vg)/kg] into the thoracic cavity of 1-day-old mice (see supplementary materials) and after 3 weeks, all organs were dissected and EGFP expression was assessed by fluorescence microscopy. EGFP expression occurred exclusively in the heart and was absent in other organs, including the brain, lung, and spleen (fig. S2). EGFP expression was present within 48 hours after virus transduction and remained robust for 12 months (figs. S2 and S3).

We next engineered 403i and control shRNAs (short hairpin RNAs), respectively designated 403i RNAi and control RNAi, into the AAV-9-cTnT-EGFP RNAi vector so that all cells expressing EGFP would also express shRNAs. To assess the efficacy of 403i shRNA in vivo, we injected

variable amounts of 403i RNAi-encoding viruses (5×10^9 , 5×10^{11} , and 5×10^{13} vg/kg) into the thoracic cavity of 1-day-old mice. Two weeks after viral transduction, total RNA extracted from each left ventricle (LV) was individually analyzed by RNA-seq (19). Sequencing reads that corresponded to *Myh6* R403Q or wild-type *Myh6* were counted and visualized using Integrative Genomics Viewer (Broad Institute, Cambridge, MA). The expression of *Myh6* was comparable in LV tissues after transduction with control RNAi (12,118 reads per million transcripts) and 403i RNAi (11,675 reads per million transcripts), indicating that the wild-type allele was not silenced in vivo. In contrast, the ratio of *Myh6* R403Q reads to *Myh6* wild-type reads varied between 403i RNAi titers. Only the highest titer (5×10^{13} vg/kg) resulted in a significant reduction (28.5%) in the relative expression of *Myh6* R403Q compared to wild-type *Myh6* transcripts ($P = 2.5 \times 10^{-5}$) (fig. S1C).

To assess the impact of silencing *Myh6* R403Q on HCM development, we injected virus encoding the 403i RNAi cassette ($n = 8$) or control

RNAi cassette ($n = 7$) into the thoracic cavity of 1-day-old male *MHC*^{403/+} mice. At 5 to 6 weeks of age, all mice were given cyclosporine A (CsA) for 3 weeks to accelerate the emergence of HCM histopathology, as described (13). Mice were serially evaluated by echocardiography; after killing, hearts were analyzed by histopathology. After CsA treatment, control RNAi-transduced mice had LV hypertrophy and severe HCM histopathology (Fig. 2A), similar to nontransduced, CsA-treated *MHC*^{403/+} mice (13). In contrast, CsA-treated *MHC*^{403/+} mice transduced with 403i RNAi did not develop HCM (Table 1). The LVWT of 403i RNAi-transduced mice (0.84 ± 0.10 mm) was significantly less than that of mice transduced with control RNAi (1.52 ± 0.25 mm, $P = 1.9 \times 10^{-5}$) and comparable to the LVWT of wild-type mice (0.74 ± 0.05 mm, n.s.) (12). Myocardial disarray (Fig. 2B) was absent, and fibrosis (Fig. 2C) was significantly reduced, in 403i RNAi-transduced mice ($0.43 \pm 0.11\%$) relative to control RNAi-transduced hypertrophic *MHC*^{403/+} mice ($2.12 \pm 0.57\%$, $P = 0.003$). QRS interval prolongation, an electrocardiographic feature of LV hypertro-

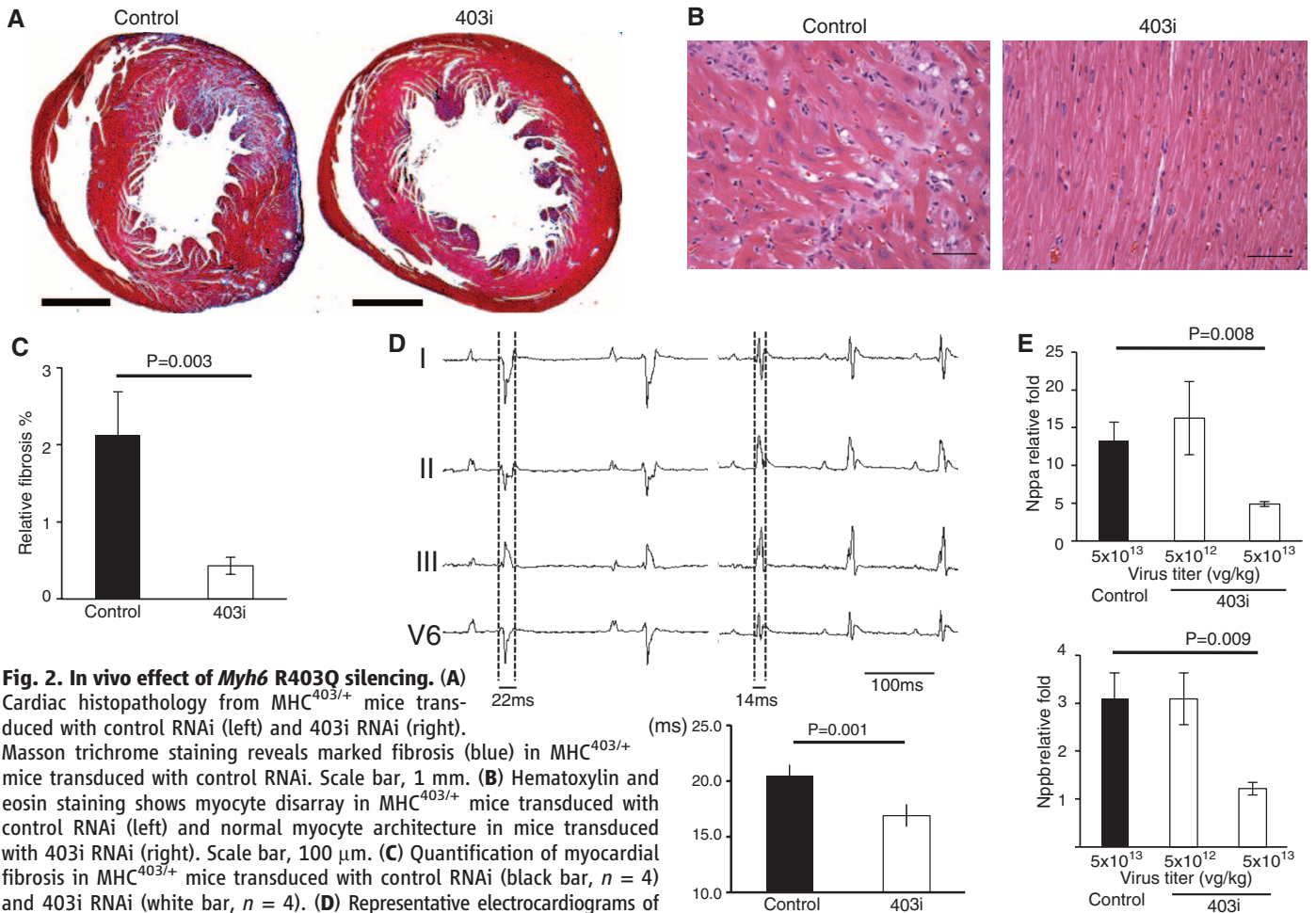


Fig. 2. In vivo effect of *Myh6* R403Q silencing. (A) Cardiac histopathology from *MHC*^{403/+} mice transduced with control RNAi (left) and 403i RNAi (right). Masson trichrome staining reveals marked fibrosis (blue) in *MHC*^{403/+} mice transduced with control RNAi. Scale bar, 1 mm. (B) Hematoxylin and eosin staining shows myocyte disarray in *MHC*^{403/+} mice transduced with control RNAi (left) and normal myocyte architecture in mice transduced with 403i RNAi (right). Scale bar, 100 μ m. (C) Quantification of myocardial fibrosis in *MHC*^{403/+} mice transduced with control RNAi (black bar, $n = 4$) and 403i RNAi (white bar, $n = 4$). (D) Representative electrocardiograms of *MHC*^{403/+} mice transduced with control RNAi (left) and 403i RNAi (right). Mice transduced with control RNAi have prolonged QRS (ventricular conduction) interval and high-voltage P-waves consistent with LV hypertrophy and atrial enlargement. Measurements of QRS intervals from mice transduced with control RNAi (black bar, $n = 5$) and 403i RNAi (white bar, $n = 6$) are shown. (E) Quantitative real-time PCR analysis of *Nppa* (left) and *Nppb* (right) expression after transduction of control RNAi (black bar) and two different doses of 403i constructs (white bar) ($n = 5$). Levels of the transcripts were normalized to transcript levels from age-matched wild-type hearts. Data are means $\pm$ SD.

phy, was present in mice transduced with control RNAi (20.5 ± 1.2 ms) but not in mice transduced with 403i RNAi (16.9 ± 1.4 ms, $P = 0.001$) (Fig. 2D). Additionally, the expression of prototypic LV hypertrophy markers *Nppa* and *Nppb* in mice transduced with control RNAi was higher than in mice transduced with 403i RNAi by a factor of 2.5 (Fig. 2E).

To assess whether the early age at transduction and/or viral titer influenced HCM development, we injected high-titer (5×10^{13} vg/kg) and low-titer (5×10^{12} vg/kg) viruses of 403i RNAi into 3-week-old MHC^{403/+} mice ($n = 5$). At 4 weeks, mice were treated with CsA for three additional weeks, followed by echocardiography to assess LVWT and diastolic (relaxation) performance [left atrial diameter normalized to the aortic root diameter (20)], which becomes abnormal early in HCM (21). Mice transduced with high viral titers of control RNAi or low viral titers of 403i RNAi had both LV hypertrophy and diastolic dysfunction (Table 2). In contrast, mice transduced with high-titer 403i RNAi virus had neither hypertrophy (LVWT = 0.72 ± 0.05 mm, $P = 1.9 \times 10^{-6}$ compared to control RNAi) nor diastolic dysfunction (left atria dimension normalized to the aortic root = 1.17 ± 0.09 , $P = 0.009$ compared to control RNAi).

Using the high-titer virus, we next investigated whether 403i RNAi transduction could alter established HCM by pretreating MHC^{403/+} mice with CsA for 3 weeks to induce hypertrophy (LVWT = 1.40 ± 0.11 mm) before viral transduction. Echocardiography assessments at 2 months after 403i RNAi ($n = 3$) transduction showed no

change in LVWT (Table 1), which suggests that the treatment was ineffective in reversing established disease.

To determine whether 403i RNAi transduction affected the pathologic LV remodeling that slowly emerges in MHC^{403/+} mice with age in the absence of CsA, we monitored LV hypertrophy in mice transduced with a single high-titer dose of 403i RNAi ($n = 5$) or control RNAi ($n = 6$) during the first day of life. At 6 months, mice transduced with control RNAi had LV hypertrophy (LVWT = 0.93 ± 0.11 mm). There was no LV hypertrophy in mice transduced with 403i RNAi (LVWT = 0.68 ± 0.09 mm, $P = 0.005$), and LVWT was indistinguishable from that of wild-type mice (LVWT = 0.74 ± 0.05 mm, n.s.).

The protective effect of the 403i RNAi vector dissipated over time. LV hypertrophy emerged in 403i RNAi-transduced mice by 11 months of age (LVWT = 0.87 ± 0.11 mm) and was comparable to that observed in control RNAi-transduced mice. The inability to fully suppress hypertrophy in MHC^{403/+} mice during later life is presumably due to diminished AAV-mediated transgene expression that occurs 7 months after transduction (22) and/or subtherapeutic 403i RNAi levels.

Finally, we considered whether a single RNAi might silence different patient-specific mutations in the same gene by targeting a nearby single-nucleotide polymorphism (SNP) that distinguished the mutant from wild-type alleles. To test this model, we produced male F₁ offspring from 129SvEv MHC^{403/+} and wild-type FVB crosses. We constructed an RNAi that targeted a 129SvEv SNP on the *Mhy6* allele (designated 129i; Fig. 1A) and

transfected this with *Mhy6* R403Q (129SvEv) or wild-type *Mhy6* (FVB) plasmids into 293T cells. The 129i RNAi decreased *Mhy6* R403Q levels by 75% and reduced wild-type *Mhy6* (FVB) by only 15% (Fig. 1C). We produced AAV-9-cTnT-EGFP-129i virus and transduced (5×10^{13} vg/kg) 1-day-old male F₁ MHC^{403/+} mice with 129i RNAi ($n = 4$) or control RNAi ($n = 5$). At 4 weeks of age, mice were treated with CsA for 2 weeks and studied by echocardiography. Control RNAi-transduced mice developed LV hypertrophy (LVWT = 1.37 ± 0.03 mm), whereas MHC^{403/+} mice transduced with 129i RNAi did not (LVWT = 0.73 ± 0.03 mm; $P = 1.6 \times 10^{-6}$) (Table 1). We conclude from these studies that one RNAi construct targeting a SNP that demarcates mutant and wild-type alleles could be used to silence distinct HCM mutations in a gene or to augment mutation-specific RNAi.

AAV-9 mediated RNAi preferentially suppresses the expression of the *Mhy6* R403Q allele in a mouse model of HCM by directly targeting the mutation or a nearby SNP. It is noteworthy that in a mouse model characterized by accelerated onset and severity of HCM, reduction in the expression levels of the mutant allele by only 28.5% (fig. S1C) was sufficient to abrogate hypertrophy and histopathologic remodeling for several months. Indeed, the LVWT in 403i-treated MHC^{403/+} mice, with or without CsA, was comparable to that in wild-type mice. On the basis of these findings and evidence for unequal expression of mutated and wild-type *MYH7* mRNAs in human HCM hearts (23), we suggest that variable penetrance and severity of HCM may reflect,

Table 1. RNAi effects on cardiac morphology and function in HCM mice.

To accelerate hypertrophic remodeling in MHC^{403/+} mice, we administered CsA for the number of weeks indicated either after RNAi transduction on day 1 (post) or for 3 weeks before RNAi transduction on day 42 (pre). Age denotes time of cardiac

evaluation; LVDD, left ventricular diastolic dimension; FS, fractional shortening. Cardiac dimensions and function with associated P values, calculated by t test, reflect comparisons to MHC^{403/+} transduced with control RNAi. Values for wild-type 129SvEv mice not treated with CsA are shown for comparison. Data are means $\pm$ SD.

CsA	RNAi	Age	<i>n</i>	LVDD (mm)	<i>P</i>	LVWT (mm)	<i>P</i>	FS (%)	<i>P</i>
3 weeks, post	Control	8 weeks	7	2.53 ± 0.41		1.52 ± 0.25		32.68 ± 6.68	
	403i	8 weeks	8	3.04 ± 0.21	0.01	0.84 ± 0.10	1.9×10^{-5}	40.37 ± 5.39	0.04
3 weeks, pre	None	6 weeks	3	2.92 ± 0.28		1.40 ± 0.11		28.38 ± 5.16	
	403i	14 weeks	3	3.50 ± 0.16	n.s.	1.42 ± 0.06	n.s.	30.61 ± 4.59	n.s.
No CsA treatment	Control	6 months	6	3.54 ± 0.27		0.93 ± 0.11		33.30 ± 4.02	
	403i	6 months	5	3.75 ± 0.21	n.s.	0.68 ± 0.09	0.005	33.42 ± 7.61	n.s.
	Control	11 months	6	3.66 ± 0.25		0.98 ± 0.16		37.8 ± 11.11	
	403i	11 months	5	3.93 ± 0.48	n.s.	0.87 ± 0.11	n.s.	36.11 ± 8.86	n.s.
2 weeks, post	Control	6 weeks	5	2.62 ± 0.20		1.37 ± 0.07		33.75 ± 4.51	
	129i	6 weeks	4	3.85 ± 0.22	0.001	0.73 ± 0.03	1.6×10^{-6}	28.21 ± 9.34	n.s.
Wild type, no CsA	None	6 months	4	3.75 ± 0.28		0.74 ± 0.05		30.01 ± 3.73	

Table 2. Viral dosage needed for RNAi effects on cardiac morphology and function in HCM mice. MHC^{403/+} mice were transduced with high-titer (5×10^{13} vg/kg) and low-titer (5×10^{12} vg/kg) RNAi on day 1 and treated with CsA for 3 weeks to accelerate hypertrophic remodeling. Left atria

(LA) dimensions normalized to the aortic root (Ao) are provided. Cardiac dimensions and function with associated P values, calculated by t test, reflect comparisons to MHC^{403/+} transduced with control RNAi. Data are means $\pm$ SD.

RNAi	Titer	LVDD (mm)	<i>P</i>	LVWT (mm)	<i>P</i>	FS (%)	<i>P</i>	LA/Ao root	<i>P</i>
Control ($n = 5$)	5×10^{13}	3.18 ± 0.24		1.34 ± 0.09		31.18 ± 7.27		1.46 ± 0.12	
403i ($n = 5$)	5×10^{13}	3.33 ± 0.15	n.s.	0.72 ± 0.05	1.9×10^{-6}	38.09 ± 4.64	n.s.	1.17 ± 0.09	0.009
403i ($n = 5$)	5×10^{12}	3.06 ± 0.34	n.s.	1.26 ± 0.26		30.33 ± 3.64		1.39 ± 0.12	

at least in part, the proportion of wild-type and mutant transcripts and proteins.

The capacity for RNAi to attenuate the expression of *Myh6* R403Q transcripts and abrogate HCM in mice raises the possibility that mutation silencing might benefit human cardiomyopathy patients. Although our study does not address the many important potential problems associated with viral-mediated gene therapy (including the potential for immune response and long-term off-target effects), some AAV protocols are sufficiently safe for human clinical trials (24). Moreover, the potential development of RNAi molecules that target allele-specific common SNPs (rather than each patient's specific mutation) could be a way to avoid the daunting challenge of producing the thousands of RNAi molecules that would be required to silence each unique HCM or dilated cardiomyopathy (DCM) mutation. There are also new opportunities to deliver gene silencing therapies to the heart. For example, the septal perforating artery can be selectively cannulated to target interventions to the interventricular septum (25), which in the majority of patients is the most profoundly hypertrophied segment in the HCM heart and can cause obstructed ventricular outflow of blood, increasing the risk of sudden death. Adaptation of this approach could facilitate prophylactic, localized silencing of HCM

mutations in this region, where its benefit could be measurable and clinically meaningful. With continued advances in viral and other delivery systems (26, 27), allele-selective silencing holds considerable potential to retard the onset and progression of HCM and other genetic cardiomyopathies.

References and Notes

1. P. Spirito, C. E. Seidman, W. J. McKenna, B. J. Maron, *N. Engl. J. Med.* **336**, 775–785 (1997).
2. C. Y. Ho, *Circulation* **125**, 1432–1438 (2012).
3. B. J. Maron, M. S. Maron, *Lancet* **381**, 242–255 (2013).
4. B. J. Maron, J. J. Doerer, T. S. Haas, D. M. Tierney, F. O. Mueller, *Circulation* **119**, 1085–1092 (2009).
5. J. G. Seidman, C. Seidman, *Cell* **104**, 557–567 (2001).
6. P. Teekakirikul, R. F. Padera, J. G. Seidman, C. E. Seidman, *J. Cell Biol.* **199**, 417–421 (2012).
7. L. R. Lopes, M. S. Rahman, P. M. Elliott, *Heart* **10.1136/heartjnl-2013-303939** (2013).
8. A. A. Geisterfer-Lowrance *et al.*, *Cell* **62**, 999–1006 (1990).
9. A. A. Geisterfer-Lowrance *et al.*, *Science* **272**, 731–734 (1996).
10. M. J. Tyska *et al.*, *Circ. Res.* **86**, 737–744 (2000).
11. D. Fatkin *et al.*, *J. Clin. Invest.* **106**, 1351–1359 (2000).
12. J. B. Kim *et al.*, *Science* **316**, 1481–1484 (2007).
13. P. Teekakirikul *et al.*, *J. Clin. Invest.* **120**, 3520–3529 (2010).
14. C. Semsarian *et al.*, *J. Clin. Invest.* **109**, 1013–1020 (2002).
15. W. K. Jones *et al.*, *J. Clin. Invest.* **98**, 1906–1917 (1996).
16. A. de Fougerolles, H. P. Vornlocher, J. Maraganore, J. Lieberman, *Nat. Rev. Drug Discov.* **6**, 443–453 (2007).
17. G. Gao, L. H. Vandenbergh, J. M. Wilson, *Curr. Gene Ther.* **5**, 285–297 (2005).
18. K. M. Prasad, Y. Xu, Z. Yang, S. T. Acton, B. A. French, *Gene Ther.* **18**, 43–52 (2011).
19. D. C. Christodoulou, J. M. Gorham, D. S. Herman, J. G. Seidman, *Curr. Protoc. Mol. Biol.*, Chapter 4, Unit 4.12 (2011).
20. R. M. Lang *et al.*, *J. Am. Soc. Echocardiogr.* **18**, 1440–1463 (2005).
21. C. Y. Ho *et al.*, *Circulation* **105**, 2992–2997 (2002).
22. L. T. Bish *et al.*, *Hum. Gene Ther.* **19**, 1359–1368 (2008).
23. S. Tripathi *et al.*, *Basic Res. Cardiol.* **106**, 1041–1055 (2011).
24. F. Mingozzi, K. A. High, *Nat. Rev. Genet.* **12**, 341–355 (2011).
25. S. Elmariah, M. A. Fifer, *Curr. Treat. Options Cardiovasc. Med.* **14**, 665–678 (2012).
26. E. Rodriguez-Lebron, H. L. Paulson, *Gene Ther.* **13**, 576–581 (2006).
27. D. Yu *et al.*, *Cell* **150**, 895–908 (2012).

Acknowledgments: We thank J. Gorham, D. Conner, and S. DePalma for technical and bioinformatic assistance, and C. Cepko for assistance with viral protocols. Supported by NIH grants U01HL098166 and R01HL084553 (J.G.S. and C.E.S.) and the Howard Hughes Medical Institute (C.E.S.). The authors (J.J., C.E.S., J.G.S., and H.W.) and Harvard Medical School plan a patent application related to the use of short hairpin RNAs as therapies for human genetic cardiomyopathies. C.E.S. and J.G.S. are founders of and own shares in MyoKardia, a biotechnology company developing small molecules that target the sarcomere for treatment of inherited cardiomyopathy.

Supplementary Materials

www.sciencemag.org/content/342/6154/111/suppl/DC1
Materials and Methods
Figs. S1 to S4

22 February 2013; accepted 3 September 2013
10.1126/science.1236921

A Thylakoid-Located Two-Pore K⁺ Channel Controls Photosynthetic Light Utilization in Plants

Luca Carraretto,¹ Elide Formentin,^{1*} Enrico Teardo,^{1*} Vanessa Checchetto,^{1,2,3,4,5} Martino Tomizioli,^{2,3,4,5} Tomas Morosinotto,¹ Giorgio Mario Giacometti,¹ Giovanni Finazzi,^{2,3,4,5†} Ildikó Szabó^{1†}

The size of the light-induced proton motive force (pmf) across the thylakoid membrane of chloroplasts is regulated in response to environmental stimuli. Here, we describe a component of the thylakoid membrane, the two-pore potassium (K⁺) channel TPK3, which modulates the composition of the pmf through ion counterbalancing. Recombinant TPK3 exhibited potassium-selective channel activity sensitive to Ca²⁺ and H⁺. In *Arabidopsis* plants, the channel is found in the thylakoid stromal lamellae. *Arabidopsis* plants silenced for the TPK3 gene display reduced growth and altered thylakoid membrane organization. This phenotype reflects an impaired capacity to generate a normal pmf, which results in reduced CO₂ assimilation and deficient nonphotochemical dissipation of excess absorbed light. Thus, the TPK3 channel manages the pmf necessary to convert photochemical energy into physiological functions.

In plants, photosynthesis converts light energy into organic carbon through the transient production of highly reactive intermediate species. Nevertheless, damage to the plant cell engine is limited because the production of reducing equivalents via the electron flow [the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)] is commensurate with energy production [via adenosine 5'-triphosphate (ATP)] for CO₂ assimilation (1). The key element bridging

the two processes is the transmembrane electrochemical proton gradient or proton motive force (pmf) (2), which is produced by electron transfer. The pmf supports ATP synthesis (2), controls the rate of electron flow [photosynthetic control (3)], and enhances the thermal dissipation of excess absorbed light [nonphotochemical quenching, NPQ (4)]. Although both components of the pmf (the proton gradient ΔpH and the electric field ΔΨ) are equally necessary for ATP synthesis (2),

the ΔpH specifically regulates photosynthetic control and NPQ by modulating both the protonation state of key regulatory proteins [e.g., (PsbS)] (5) and the rate of electron flow in the cytochrome b₆f complex (3). It is therefore assumed that the size of the ΔpH must be regulated in response to environmental stimuli through a still-elusive mechanism (6). The present work identifies a new component of thylakoid membranes, the two-pore potassium (K⁺) channel TPK3, which regulates the transmembrane ΔpH through ion counterbalancing in the thylakoid membrane and, thus, controls the nature of the pmf.

The plant TPK channel (tandem-pore K⁺ channel) family represents the counterpart of the animal leak TWIK/TREK channels (7). Among the five members of this family, AtTPK1 of *A. thaliana* was the first to be identified through *in silico* searches. Vacuolar TPK1 affects germination, seedling growth, and stomatal movement (8) by mediating intracellular K⁺ homeostasis (9, 10). AtTKP4, which is targeted to the plasma

¹Department of Biology, University of Padua, viale Giuseppe Colombo 3, 35121 Padua, Italy. ²Centre National Recherche Scientifique, Unité Mixte Recherche 5168, Laboratoire Physiologie Cellulaire et Végétale, F-38054 Grenoble, France. ³Commissariat à l'Energie Atomique et Energies Alternatives, l'Institut de Recherches en Technologies et Sciences pour le Vivant, F-38054 Grenoble, France. ⁴Université Grenoble Alpes, F-38041 Grenoble, France. ⁵Institut National Recherche Agronomique, USC 1359, F-38054 Grenoble, France.

*These authors contributed equally to this work.

†Corresponding author. E-mail: ildi@cv.bio.unipd.it or ildi.ko.szabo@unipd.it (I.S.); and giovanni.finazzi@cea.fr (G.F.)



A Thylakoid-Located Two-Pore K⁺ Channel Controls Photosynthetic Light Utilization in Plants

Luca Carraretto *et al.*

Science **342**, 114 (2013);

DOI: 10.1126/science.1242113

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/114.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/09/04/science.1242113.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/114.full.html#related>

This article **cites 42 articles**, 15 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/114.full.html#ref-list-1>

at least in part, the proportion of wild-type and mutant transcripts and proteins.

The capacity for RNAi to attenuate the expression of *Myh6* R403Q transcripts and abrogate HCM in mice raises the possibility that mutation silencing might benefit human cardiomyopathy patients. Although our study does not address the many important potential problems associated with viral-mediated gene therapy (including the potential for immune response and long-term off-target effects), some AAV protocols are sufficiently safe for human clinical trials (24). Moreover, the potential development of RNAi molecules that target allele-specific common SNPs (rather than each patient's specific mutation) could be a way to avoid the daunting challenge of producing the thousands of RNAi molecules that would be required to silence each unique HCM or dilated cardiomyopathy (DCM) mutation. There are also new opportunities to deliver gene silencing therapies to the heart. For example, the septal perforating artery can be selectively cannulated to target interventions to the interventricular septum (25), which in the majority of patients is the most profoundly hypertrophied segment in the HCM heart and can cause obstructed ventricular outflow of blood, increasing the risk of sudden death. Adaptation of this approach could facilitate prophylactic, localized silencing of HCM

mutations in this region, where its benefit could be measurable and clinically meaningful. With continued advances in viral and other delivery systems (26, 27), allele-selective silencing holds considerable potential to retard the onset and progression of HCM and other genetic cardiomyopathies.

References and Notes

- P. Spirito, C. E. Seidman, W. J. McKenna, B. J. Maron, *N. Engl. J. Med.* **336**, 775–785 (1997).
- C. Y. Ho, *Circulation* **125**, 1432–1438 (2012).
- B. J. Maron, M. S. Maron, *Lancet* **381**, 242–255 (2013).
- B. J. Maron, J. J. Doerer, T. S. Haas, D. M. Tierney, F. O. Mueller, *Circulation* **119**, 1085–1092 (2009).
- J. G. Seidman, C. Seidman, *Cell* **104**, 557–567 (2001).
- P. Teekakirikul, R. F. Padera, J. G. Seidman, C. E. Seidman, *J. Cell Biol.* **199**, 417–421 (2012).
- L. R. Lopes, M. S. Rahman, P. M. Elliott, *Heart* **10.1136/heartjnl-2013-303939** (2013).
- A. A. Geisterfer-Lowrance *et al.*, *Cell* **62**, 999–1006 (1990).
- A. A. Geisterfer-Lowrance *et al.*, *Science* **272**, 731–734 (1996).
- M. J. Tyska *et al.*, *Circ. Res.* **86**, 737–744 (2000).
- D. Fatkin *et al.*, *J. Clin. Invest.* **106**, 1351–1359 (2000).
- J. B. Kim *et al.*, *Science* **316**, 1481–1484 (2007).
- P. Teekakirikul *et al.*, *J. Clin. Invest.* **120**, 3520–3529 (2010).
- C. Semsarian *et al.*, *J. Clin. Invest.* **109**, 1013–1020 (2002).
- W. K. Jones *et al.*, *J. Clin. Invest.* **98**, 1906–1917 (1996).
- A. de Fougerolles, H. P. Vornlocher, J. Maraganore, J. Lieberman, *Nat. Rev. Drug Discov.* **6**, 443–453 (2007).
- G. Gao, L. H. Vandenbergh, J. M. Wilson, *Curr. Gene Ther.* **5**, 285–297 (2005).
- K. M. Prasad, Y. Xu, Z. Yang, S. T. Acton, B. A. French, *Gene Ther.* **18**, 43–52 (2011).
- D. C. Christodoulou, J. M. Gorham, D. S. Herman, J. G. Seidman, *Curr. Protoc. Mol. Biol.*, Chapter 4, Unit 4.12 (2011).
- R. M. Lang *et al.*, *J. Am. Soc. Echocardiogr.* **18**, 1440–1463 (2005).
- C. Y. Ho *et al.*, *Circulation* **105**, 2992–2997 (2002).
- L. T. Bish *et al.*, *Hum. Gene Ther.* **19**, 1359–1368 (2008).
- S. Tripathi *et al.*, *Basic Res. Cardiol.* **106**, 1041–1055 (2011).
- F. Mingozzi, K. A. High, *Nat. Rev. Genet.* **12**, 341–355 (2011).
- S. Elmariah, M. A. Fifer, *Curr. Treat. Options Cardiovasc. Med.* **14**, 665–678 (2012).
- E. Rodriguez-Lebron, H. L. Paulson, *Gene Ther.* **13**, 576–581 (2006).
- D. Yu *et al.*, *Cell* **150**, 895–908 (2012).

Acknowledgments: We thank J. Gorham, D. Conner, and S. DePalma for technical and bioinformatic assistance, and C. Cepko for assistance with viral protocols. Supported by NIH grants U01HL098166 and R01HL084553 (J.G.S. and C.E.S.) and the Howard Hughes Medical Institute (C.E.S.). The authors (J.J., C.E.S., J.G.S., and H.W.) and Harvard Medical School plan a patent application related to the use of short hairpin RNAs as therapies for human genetic cardiomyopathies. C.E.S. and J.G.S. are founders of and own shares in MyoKardia, a biotechnology company developing small molecules that target the sarcomere for treatment of inherited cardiomyopathy.

Supplementary Materials

www.sciencemag.org/content/342/6154/111/suppl/DC1
Materials and Methods
Figs. S1 to S4

22 February 2013; accepted 3 September 2013
10.1126/science.1236921

A Thylakoid-Located Two-Pore K⁺ Channel Controls Photosynthetic Light Utilization in Plants

Luca Carraretto,¹ Elide Formentin,^{1*} Enrico Teardo,^{1*} Vanessa Checchetto,^{1,2,3,4,5} Martino Tomizoli,^{2,3,4,5} Tomas Morosinotto,¹ Giorgio Mario Giacometti,¹ Giovanni Finazzi,^{2,3,4,5†} Ildikó Szabó^{1†}

The size of the light-induced proton motive force (pmf) across the thylakoid membrane of chloroplasts is regulated in response to environmental stimuli. Here, we describe a component of the thylakoid membrane, the two-pore potassium (K⁺) channel TPK3, which modulates the composition of the pmf through ion counterbalancing. Recombinant TPK3 exhibited potassium-selective channel activity sensitive to Ca²⁺ and H⁺. In *Arabidopsis* plants, the channel is found in the thylakoid stromal lamellae. *Arabidopsis* plants silenced for the TPK3 gene display reduced growth and altered thylakoid membrane organization. This phenotype reflects an impaired capacity to generate a normal pmf, which results in reduced CO₂ assimilation and deficient nonphotochemical dissipation of excess absorbed light. Thus, the TPK3 channel manages the pmf necessary to convert photochemical energy into physiological functions.

In plants, photosynthesis converts light energy into organic carbon through the transient production of highly reactive intermediate species. Nevertheless, damage to the plant cell engine is limited because the production of reducing equivalents via the electron flow [the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)] is commensurate with energy production [via adenosine 5'-triphosphate (ATP)] for CO₂ assimilation (1). The key element bridging

the two processes is the transmembrane electrochemical proton gradient or proton motive force (pmf) (2), which is produced by electron transfer. The pmf supports ATP synthesis (2), controls the rate of electron flow [photosynthetic control (3)], and enhances the thermal dissipation of excess absorbed light [nonphotochemical quenching, NPQ (4)]. Although both components of the pmf (the proton gradient ΔpH and the electric field ΔΨ) are equally necessary for ATP synthesis (2),

the ΔpH specifically regulates photosynthetic control and NPQ by modulating both the protonation state of key regulatory proteins [e.g., (PsbS)] (5) and the rate of electron flow in the cytochrome b₆f complex (3). It is therefore assumed that the size of the ΔpH must be regulated in response to environmental stimuli through a still-elusive mechanism (6). The present work identifies a new component of thylakoid membranes, the two-pore potassium (K⁺) channel TPK3, which regulates the transmembrane ΔpH through ion counterbalancing in the thylakoid membrane and, thus, controls the nature of the pmf.

The plant TPK channel (tandem-pore K⁺ channel) family represents the counterpart of the animal leak TWIK/TREK channels (7). Among the five members of this family, AtTPK1 of *A. thaliana* was the first to be identified through *in silico* searches. Vacuolar TPK1 affects germination, seedling growth, and stomatal movement (8) by mediating intracellular K⁺ homeostasis (9, 10). AtTKP4, which is targeted to the plasma

¹Department of Biology, University of Padua, viale Giuseppe Colombo 3, 35121 Padua, Italy. ²Centre National Recherche Scientifique, Unité Mixte Recherche 5168, Laboratoire Physiologie Cellulaire et Végétale, F-38054 Grenoble, France. ³Commissariat à l'Energie Atomique et Energies Alternatives, l'Institut de Recherches en Technologies et Sciences pour le Vivant, F-38054 Grenoble, France. ⁴Université Grenoble Alpes, F-38041 Grenoble, France. ⁵Institut National Recherche Agronomique, USC 1359, F-38054 Grenoble, France.

*These authors contributed equally to this work.

†Corresponding author. E-mail: ildi@cv.bio.unipd.it or ildi.ko.szabo@unipd.it (I.S.); and giovanni.finazzi@cea.fr (G.F.)

membrane, is a voltage-independent, open rectifier, calcium- and proton-sensitive K^+ channel (11). The biophysical properties and physiological roles of AtTPK2, 3, and 5 (12–14) remain unknown. However, pore domain-swapping experiments have highlighted a similar selectivity and instantaneous activity of chimeric channels (between AtTPK2, 3, and 5 and AtTPK4) compared with TPK4 (15). AtTPK1, 2, and 5 restored growth of a K^+ uptake-deficient *Escherichia coli* mutant (16).

Activity of AtTPK3 was evaluated in planar lipid bilayer experiments by using recombinant purified AtTPK3, given that patch clamping of the *Arabidopsis* thylakoid membrane was not feasible. The apparent molecular mass (51 kD) of AtTPK3 expressed in *E. coli* as a fusion protein with an N-terminal hexahistidine (His_6) tag is in good agreement with the predicted weight (54 kD) (fig. S1A). The eluted protein was recognized by both a monoclonal antibody against TPK3 (17) and a more general antibody raised against the highly conserved selectivity filter region of K^+ channels (fig. S1B), which indicates successful purification of AtTPK3. We recorded channel activity in symmetrical potassium gluconate solution (250 mM), in the presence of calcium, by using gluconate as an impermeant anion (Fig. 1A).

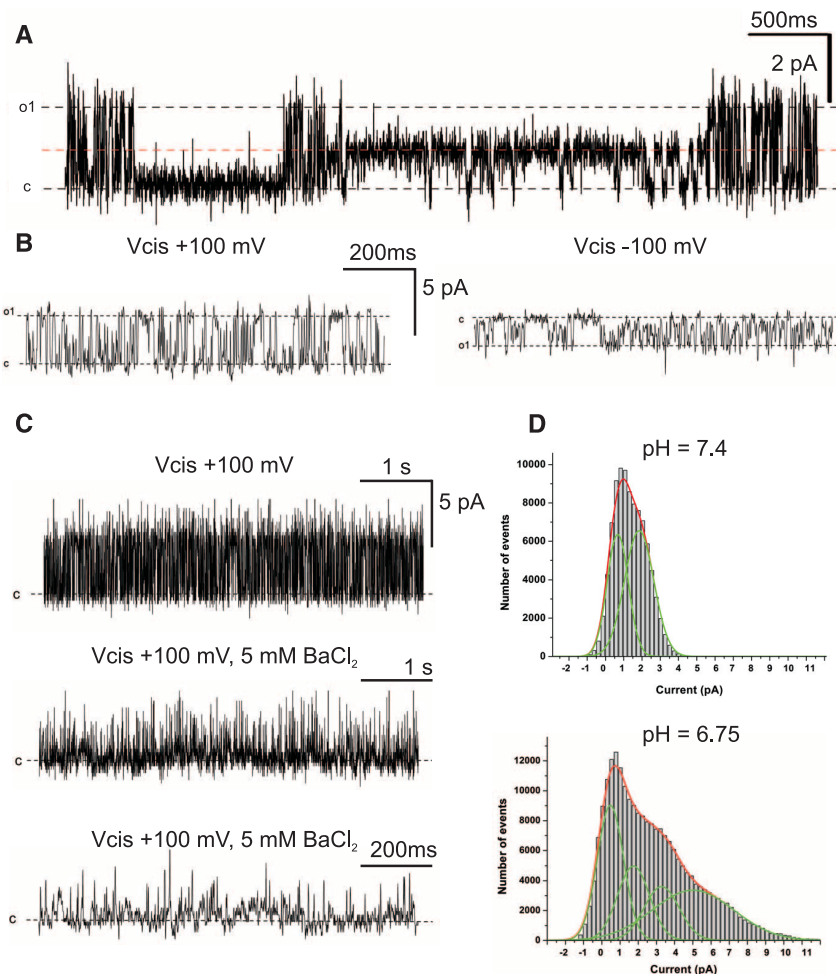
The current-voltage relation [single-channel amplitude (i) as a function of voltage (V), the i - V curve] showed a conductance of 35 pS (fig. S2A). Measurements performed in asymmetric ionic conditions (500/100 mM KCl) (Fig. 1B) led to the generation of an i - V curve (fig. S2B) yielding a reversal potential (E_{rev}) of -28 mV (the theoretical E_{rev} for a perfectly selective K^+ channel is -36 mV), which confirmed a preference for potassium versus chloride. In accordance with the presence of an EF hand in the protein, calcium was required for channel activity (fig. S2C). Studies with classical K^+ channel inhibitors confirmed the nature of AtTPK3. Barium added to the cis side resulted in a fast block at a 5 mM concentration (Fig. 1C), whereas 50 mM tetraethylammonium (TEA^+) did not affect channel activity (fig. S2D). AtTPK1 can be activated by protons (8). Activity of AtTPK3 also increased when the cis compartment was acidified, as indicated by an increased current level in the resultant amplitude histogram (Fig. 1D). Overall, the main biophysical properties of the AtTPK3 activity are in accordance with those of TPKs (7, 8).

Using an AtTPK3::DsRed2 fusion protein, we localized AtTPK3 in the chloroplast of *Arabidopsis* leaves (fig. S3A), and subchloroplast localization in wild-type (wt) plants by immu-

noblotting with an antibody against TPK3 revealed that this protein is located in the stroma lamellae (Fig. 2A and fig. S3B), the same compartment where the chloroplast H^+ carrier (the ATP synthase CF_0F_1) is found. To investigate the physiological role of AtTPK3, *Arabidopsis* knockdown (KD) lines were generated because knockout transfer DNA insertion lines could not be found in existing mutant banks (17). Three genetically independent lines were obtained where silencing of the gene was confirmed by transcript analysis (fig. S4A) and the Western blot of thylakoids (fig. S4B). Seedlings of wt and silenced plants grown on agar plates did not show any differences in germination (fig. S5). Silenced plants grown in soil under a light regime consisting of a light-dark 12:12 photoperiod at a light intensity of $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ showed no difference with respect to wt (Fig. 2B), which ruled out any detrimental effect of the silencing construct and of the applied procedure. However, when the light intensity was increased to $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the silenced plants exhibited a decreased rosette size (Fig. 2B and fig. S6, A and B) and an enhanced accumulation of anthocyanins (fig. S6C) but comparable amounts of other photosynthetic pigments (table S1) compared with wt. No significant differences

Fig. 1. Recombinant TPK3 induces barium-sensitive channel activity in planar lipid bilayers.

(A) Representative current trace showing the transition to a half-conductance substate (red line) recorded at 100 mV in 250 mM K gluconate. (B) Current traces recorded in an asymmetric KCl solution at the indicated voltages (cis: 500 mM KCl; trans: 100 mM KCl). The open probability was 0.65 at -100 mV and 0.57 at $+100$ mV (from 120-s trace). (C) Ionic conditions are as in (B). The addition of 5 mM barium to the cis side resulted in a fast block of the activity (middle trace). The bottom trace indicates the activity on an extended time scale. Closed state is indicated by dashed line. (D) Decreasing the pH from 7.4 to 6.75 increased activity, as observed from the amplitude histograms, obtained from current traces recorded in symmetrical K gluconate at $+50$ mV. Fitting was done using Origin software. Peak values yield conductance values in the range of 29 to 35 pS. All of the presented data are representative of at least four experiments.



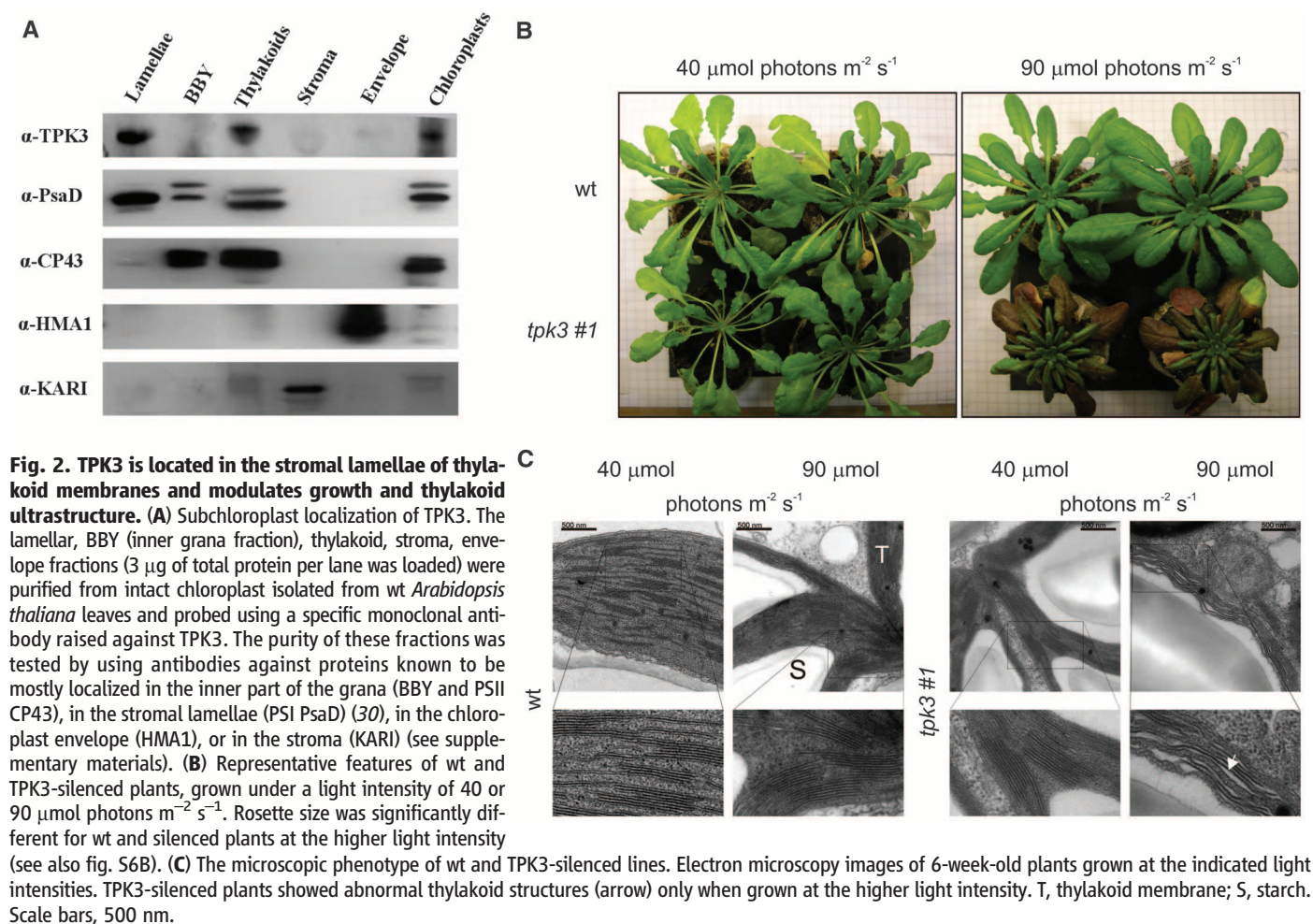


Fig. 2. TPK3 is located in the stromal lamellae of thylakoid membranes and modulates growth and thylakoid ultrastructure. (A) Subchloroplast localization of TPK3. The lamellar, BBY (inner grana fraction), thylakoid, stroma, envelope fractions (3 μg of total protein per lane was loaded) were purified from intact chloroplast isolated from wt *Arabidopsis thaliana* leaves and probed using a specific monoclonal antibody raised against TPK3. The purity of these fractions was tested by using antibodies against proteins known to be mostly localized in the inner part of the grana (BBY and PSII CP43), in the stromal lamellae (PSI PsaD) (30), in the chloroplast envelope (HMA1), or in the stroma (KARI) (see supplementary materials). **(B)** Representative features of wt and TPK3-silenced plants, grown under a light intensity of 40 or 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Rosette size was significantly different for wt and silenced plants at the higher light intensity (see also fig. S6B). **(C)** The microscopic phenotype of wt and TPK3-silenced lines. Electron microscopy images of 6-week-old plants grown at the indicated light intensities. TPK3-silenced plants showed abnormal thylakoid structures (arrow) only when grown at the higher light intensity. T, thylakoid membrane; S, starch. Scale bars, 500 nm.

were found between wt plants and plants that were subjected to the same transformation protocol but were not completely silenced (figs. S4A and S7A). In AtTPK3-silenced plants, no change in the thylakoid structure was observed at 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, although the organization of the photosynthetic membranes was compromised in all three silenced lines under a light intensity of 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 2C and fig. S7B). This thylakoid disorganization could reflect an altered composition of the photosynthetic chain. However, we found no differences at the level of the main components of the photosynthetic apparatus, including photosystem I (PSI), PSII, the ATP synthase, and the cytochrome b_6f complexes (fig. S8A). Nondenaturing Deriphat polyacrylamide gel electrophoresis (18) analysis of thylakoids solubilized with 0.6% α -dodecyl-maltoside confirmed the intactness and the similar relative abundance of the photosynthetic complexes and supercomplexes in the different genotypes (fig. S8B). We note, however, that the phosphorylation pattern of photosynthetic membranes in the light was different in wt and silenced plants lines at 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (fig. S8C).

The observed mutant phenotype should stem from changes in the photosynthetic activity rather

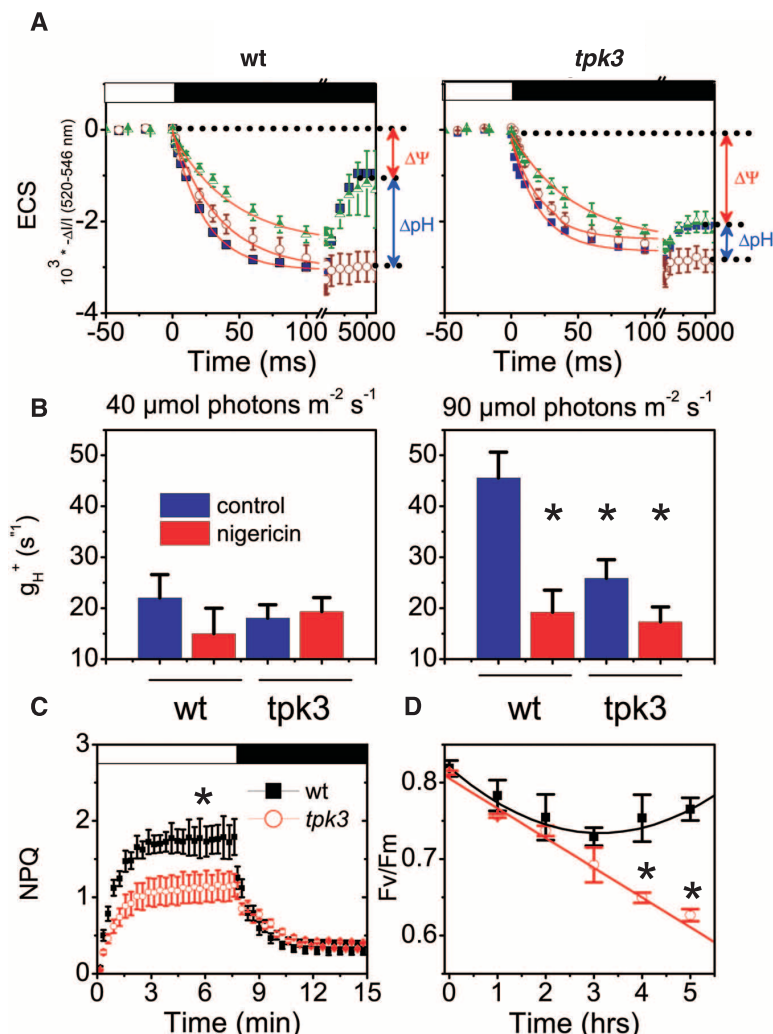
than from differences in the thylakoid protein composition. Because of the central role of the pmf in regulating light utilization in oxygenic photosynthesis and its potential sensitivity to changes in ion fluxes, we addressed possible differences in the pmf arising as a consequence of the mutation. We compared wt and mutant lines grown at a light intensity of either 40 or 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, taking advantage of the relation between pmf and the electrochromic shift (ECS). This signal stems from a shift in the absorption spectrum of specific pigments embedded in the thylakoid membranes upon exposure to the $\Delta\Psi$ of the pmf because of the Stark effect (2). Under continuous illumination, the extent of the ECS signal (Fig. 3A) reflects the amplitude of the pmf (19). Moreover, the rapid decay in the signal reflects H^+ leakage through ATP synthase (g_{H^+}), which can be evaluated by fitting the kinetics with a first-order decay (Fig. 3, A and B, red lines). On a longer time scale, an inversion of the ECS is seen, which has been interpreted as a transient inversion of the membrane potential to compensate for the slower relaxation of the ΔpH (20). Thus, the amplitude of this inverted signal can be taken as an indicator of the size of the light-stimulated ΔpH . Again, no differences were found

between the two genotypes under low light. However, under a higher-intensity light, the putative amplitude of the ΔpH and the proton conductivity of the membranes (g_{H^+}) were reduced in the silenced plants (Fig. 3B), whereas the overall size of the pmf was only slightly diminished. The consequences of the mutation were similar to those induced by the H^+/K^+ exchanger nigericin (wine-colored circles in Fig. 3A), which was used here as a control to fully suppress the ΔpH without affecting the $\Delta\Psi$. The moderate effect on the g_{H^+} in the silenced lines corroborates the notion that both the ΔpH and the $\Delta\Psi$ are able to fuel the H^+ flux through the ATP synthase (2). Indeed, although the relative amplitude of the ΔpH and the $\Delta\Psi$ is very different for the wt and silenced lines, their sum is weakly decreased in the silenced plants. This likely explains the moderate effect of the lack of the channel on g_{H^+} .

We conclude that TPK3-silenced plants exhibit a reduced (although not abolished) capacity to generate a ΔpH , while maintaining a higher electric potential in light than in the wt. This result suggests that the TPK3-mediated K^+ efflux from the lumen is required for the regulation of the transmembrane electrical potential, the enhancement of the pH gradient for ATP synthesis,

Fig. 3. Functional characterization of wt and TPK3 plants grown at a light intensity of 40 or 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

(A) Dark relaxation of the ECS signal after illumination (white box) with $1100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Triangles, $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; squares, $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$; circles, nigericin ($10 \mu\text{M}$). Although the relative amplitude of ΔpH and the $\Delta\Psi$ is very different between the lines, their sum is only slightly decreased in the silenced plants (<20%). (B) H^+ conductivity through ATP synthase (g_{H^+}) in the presence and absence of the H^+/K^+ exchanger nigericin. Differences are statistically significant ($P < 0.05$) between wt and *tpk3* plants grown at $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Proton permeability was evaluated as $g_{\text{H}^+} = 1/\tau$ after fitting the decay kinetics with a single exponential [(A), red lines]. Fitting the traces with two exponentials did not improve the quality of the fit; means $\pm$ SEM ($n = 4$ to 10). (C) NPQ capacity; NPQ was estimated according to the formula for calculating fluorescence yield $F_m - F_m/F_m'$ (28); means $\pm$ SEM ($n = 20$ to 25). (D) The ratio of variable to maximum fluorescence (F_v/F_m) values for wt and TPK3-silenced lines after photoinhibition. Plants, grown at a light intensity of $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, were exposed to $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the indicated times. Mean F_v/F_m values $\pm$ SEM were collected after 20 min of dark adaptation [$n = 75$ (25 plants per genotype)]. All experiments of this figure refer to data from all three silenced lines.



the regulation of electron flow, and pH-mediated photoprotective responses (21). To investigate this possibility, we measured the capacity of the silenced plants to thermally dissipate excess light through nonphotochemical quenching (NPQ) in the antenna complexes using a fluorescence set-up to image whole plants. We detected significantly reduced NPQ in mutant plants shifted from low to higher light compared with wt plants (Fig. 3C), confirming that the partitioning between the ΔpH and the $\Delta\Psi$ is a critical parameter for light acclimation in plants (22, 23). It is noteworthy that putrescine, a diamine, which increases the $\Delta\Psi/\Delta\text{pH}$ ratio, reduces NPQ and induces a modification of the thylakoid structure (24) in wt plants, similarly to what we observed here. Decreased NPQ often paves the way to light sensitivity, as observed in TPK3-silenced plants during experiments where photoinhibition was measured in intact plants (Fig. 3D). The difference between the two genotypes disappeared progressively when the plants were shifted from 40 to 300 and 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (fig. S9), i.e., at light intensities where photoinhibition was also visible in the wt. This confirms the higher photosensitivity of the mutant. The observed over-

reduction of the electron flow chain in silenced plants (fig. S9) could also explain the observed decreased phosphorylation of light-harvesting complexes at $90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in these plants (fig. S8C), due to enhanced redox inhibition of membrane-bound kinases (25) by the reduced electron carriers.

In photosynthetic membranes, photophosphorylation is driven by the electrochemical proton gradient. It is acknowledged that the light-induced proton influx must be electrically balanced by ion fluxes in the opposite direction. Previous works have demonstrated Cl^- channel (26) and K^+ channel (27) activities in the thylakoid membrane and have hypothesized a role for these channels in such counterbalance. Our findings show that AtTPK3 (the sole TPK channel in the thylakoids) modulates the partitioning of the pmf between the $\Delta\Psi$ and ΔpH in chloroplasts in vivo at physiological light intensities. By controlling the size and composition of the pmf, AtTPK3 directly affects light utilization and dissipation and thereby influences chloroplast ultrastructure and plant fitness. TPK3 is therefore the missing element responsible for the electro-neutrality of proton pumping in this organelle,

as originally conceived under the chemiosmotic theory.

References and Notes

1. J. F. Allen, *Cell* **110**, 273–276 (2002).
2. H. T. Witt, *Biochim. Biophys. Acta* **505**, 355–427 (1979).
3. D. S. Bendall, *Biochim. Biophys. Acta* **683**, 119–151 (1982).
4. C. A. Wraight, A. R. Crofts, *Eur. J. Biochem.* **17**, 319–327 (1970).
5. Z. Li, S. Wakao, B. B. Fischer, K. K. Niyogi, *Annu. Rev. Plant Biol.* **60**, 239–260 (2009).
6. D. M. Kramer, J. A. Cruz, A. Kanazawa, *Trends Plant Sci.* **8**, 27–32 (2003).
7. P. Enyedi, G. Cziráj, *Physiol. Rev.* **90**, 559–605 (2010).
8. A. Gobert, S. Isayenkov, C. Voelker, K. Czempinski, F. J. Maathuis, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10726–10731 (2007).
9. G. Schönknecht *et al.*, *FEBS Lett.* **511**, 28–32 (2002).
10. H. Bihler *et al.*, *Plant Physiol.* **139**, 417–424 (2005).
11. D. Becker *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15621–15626 (2004).
12. C. Voelker, D. Schmidt, B. Mueller-Roeber, K. Czempinski, *Plant J.* **48**, 296–306 (2006).
13. M. Dunkel *et al.*, *Mol. Plant* **1**, 938–949 (2008).
14. C. Voelker *et al.*, *Plant Biol. (Stuttgart)* **12** (Suppl. 1), 56–63 (2010).
15. D. Marcel, T. Müller, R. Hedrich, D. Geiger, *FEBS Lett.* **584**, 2433–2439 (2010).

16. S. Isayenkov, F. J. Maathuis, *Plant Signal. Behav.* **8**, e24665 (2013).
17. M. Zanetti *et al.*, *PLOS ONE* **5**, e10118 (2010).
18. S. de Bianchi *et al.*, *Plant Cell* **23**, 2659–2679 (2011).
19. B. Bailleul, P. Cardol, C. Breyton, G. Finazzi, *Photosynth. Res.* **106**, 179–189 (2010).
20. J. A. Cruz, C. A. Sacksteder, A. Kanazawa, D. M. Kramer, *Biochemistry* **40**, 1226–1237 (2001).
21. S. Eberhard, G. Finazzi, F. A. Wollman, *Annu. Rev. Genet.* **42**, 463–515 (2008).
22. N. E. Ioannidis, J. A. Cruz, K. Kotzabasis, D. M. Kramer, *PLOS ONE* **7**, e29864 (2012).
23. T. J. Avenso, J. A. Cruz, D. M. Kramer, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5530–5535 (2004).
24. N. V. Paramonova, N. I. Shevyakova, V. V. Kuznetsov, *Russ. J. Plant Physiol.* **51**, 86–96 (2004).
25. E. Rintamäki, P. Martinsuo, S. Pursiheimo, E. M. Aro, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11644–11649 (2000).
26. G. Schönknecht, R. Hedrich, W. Junge, K. Raschke, *Nature* **336**, 589–592 (1988).
27. M. Tester, M. R. Blatt, *Plant Physiol.* **91**, 249–252 (1989).

28. K. Maxwell, G. N. Johnson, *J. Exp. Bot.* **51**, 659–668 (2000).

Acknowledgments: The authors are grateful to M. Zoratti and N. La Rascio for useful discussion and to J. Barber, N. Uozumi, P. Joliot, and N. Rolland for critical reading of the manuscript. We thank A. Spena for the pBIN RoC plasmid, L. Leanza for help with antibody production, and M. Simonetti for help with artwork. We thank Italy's PRIN (Research Projects of National Interest) (2010CSJX4F_005 to I.S.) and European Molecular Biology Organization Young Investigator Program for financial support (to I.S.) and the Aldo Gini Program for a short-term grant to V.C. to work in the laboratory of G.F.; the Ph.D. fellowship for L.C. was provided by the Italian Ministry of Education (L170). M.T. was supported by the French National Agency (ANR-2010-GENOM-BTV-002-01 Chloro-Type). G.F. was funded by the French National Agency "Project PhytAdapt" (ANR-NT09_567009), the Marie Curie Initial Training Network "AccliPhot" (FP7-PEOPLE-2012-ITN, grant agreement number 316427), and the Labex GRAL (Grenoble Alliance for Integrated Structural Cell Biology) grants. Author contributions: expression and purification of ATPK3: L.C.

and E.T.; electrophysiology: L.C., E.T., and I.S.; localization: L.C., E.F., and M.T.; silencing: L.C. and E.F.; pigment analysis: L.C. and T.M.; analysis of visible phenotype: L.C., T.M., I.S., and G.F.; electron microscopy and transmission electron microscopy service: L.C.; pmf measurements: M.T., V.C., and G.F. The study was designed by I.S., G.F., and G.M.G.; the paper was written by I.S. and G.F. This paper is dedicated to G. Franchin and E. Vaccher at Centro di Riferimento Oncologico (CRO), Aviano. Authors declare no conflict of interest. Additional data are in the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6154/114/suppl/DC1

Materials and Methods

Figs. S1 to S9

Tables S1 and S2

References (29–42)

18 June 2013; accepted 27 August 2013

Published online 5 September 2013;

10.1126/science.1242113

Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways

Arne Weiberg,^{1,2,3*} Ming Wang,^{1,2,3*} Feng-Mao Lin,⁴ Hongwei Zhao,^{1,2,3†} Zhihong Zhang,^{1,2,3,5} Isgouhi Kaloshian,^{2,3,6} Hsien-Da Huang,^{4,7} Hailing Jin^{1,2,3‡}

Botrytis cinerea, the causative agent of gray mold disease, is an aggressive fungal pathogen that infects more than 200 plant species. Here, we show that some *B. cinerea* small RNAs (Bc-sRNAs) can silence *Arabidopsis* and tomato genes involved in immunity. These Bc-sRNAs hijack the host RNA interference (RNAi) machinery by binding to *Arabidopsis* Argonaute 1 (AGO1) and selectively silencing host immunity genes. The *Arabidopsis ago1* mutant exhibits reduced susceptibility to *B. cinerea*, and the *B. cinerea dcl1 dcl2* double mutant that can no longer produce these Bc-sRNAs displays reduced pathogenicity on *Arabidopsis* and tomato. Thus, this fungal pathogen transfers "virulent" sRNA effectors into host plant cells to suppress host immunity and achieve infection, which demonstrates a naturally occurring cross-kingdom RNAi as an advanced virulence mechanism.

Botrytis cinerea is a fungal pathogen that infects almost all vegetable and fruit crops and annually causes \$10 billion to \$100 billion in losses worldwide. With its broad host range, *B. cinerea* is a useful model for studying the pathogenicity of aggressive fungal pathogens. Many pathogens of plants and animals deliver effectors into host cells to suppress host immunity (1–4). All the pathogen effectors studied so

far are proteins. We found that small RNA (sRNA) molecules derived from *B. cinerea* can act as effectors to suppress host immunity.

sRNAs induce gene silencing by binding to Argonaute (AGO) proteins and directing the RNA-induced silencing complex (RISC) to genes with complementary sequences. sRNAs from both plant and animal hosts have been recognized as regulators in host-microbial interaction (5–8). Although sRNAs are also present in various fungi and oomycetes, including many pathogens (9–14), it has not been clear whether they regulate host-pathogen interaction.

To explore the role of *B. cinerea* sRNAs in pathogenicity, we profiled sRNA libraries prepared from *B. cinerea* (strain B05.10)–infected *Arabidopsis thaliana* Col-0 leaves collected at 0, 24, 48, and 72 hours after inoculation and from *B. cinerea*–infected *Solanum lycopersicum* (tomato) leaves and fruits at 0, 24, and 72 hours after inoculation. sRNA libraries prepared from *B. cinerea* mycelia, conidiospores, and total biomass after 10 days of culture were used as controls. By using

100 normalized reads per million *B. cinerea* sRNA reads as a cutoff, we identified a total of 832 sRNAs that were present in both *B. cinerea*–infected *Arabidopsis* and *S. lycopersicum* libraries and had more reads in these libraries than in the cultured *B. cinerea* libraries, with sequences exactly matching the *B. cinerea* B05.10 genome (15) but not *Arabidopsis* or *S. lycopersicum* genomes or cDNA (tables S1 to S3). The closest sequence matches in *Arabidopsis* or *S. lycopersicum* contained a minimum of two mismatches. Among them, 27 had predicted microRNA (miRNA)–like precursor structures. A similar number of miRNA-like sRNAs were found in *Sclerotinia sclerotiorum* (9). We found that 73 Bc-sRNAs could target host genes in both *Arabidopsis* and *S. lycopersicum* under stringent target prediction criteria (tables S3). Among them, 52 were derived from six retrotransposon long terminal repeats (LTR) loci in the *B. cinerea* genome, 13 were from intergenic regions of 10 loci, and eight were mapped to five protein-coding genes.

Some of the predicted plant targets, such as mitogen-activated protein kinases (MAPKs), are likely to function in plant immunity. To test whether Bc-sRNAs could indeed suppress host genes during infection, three Bc-sRNAs (Bc-siR3.1, Bc-siR3.2, and Bc-siR5) were selected for further characterization (table S2). These Bc-sRNAs were among the most abundant sRNAs that were 21 nucleotides (nt) in length and had potential targets likely to be involved in plant immunity in both *Arabidopsis* and *S. lycopersicum*. These sRNAs were also enriched after infection (Fig. 1, A and B; fig. S1; and table S2) and were the major sRNA products from their encoding loci, LTR retrotransposons (fig. S1). Bc-siR3.1 and Bc-siR3.2 were derived from the same locus with a 4-nt shift in sequence.

To determine whether Bc-sRNAs could trigger silencing of host genes, we examined the transcript levels of the predicted target genes after *B. cinerea* infection. The following *Arabidopsis* genes were targeted in the coding regions and were suppressed after *B. cinerea* infection: *mitogen activated protein kinase 2* (MPK2) and *MPK1*, which are targeted

¹Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. ²Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA. ³Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA. ⁴Department of Biological Science and Technology, National Chiao Tung University, Hsin-Chu 300, Taiwan. ⁵College of Horticulture, Shenyang Agricultural University, Shenyang, Liaoning 110866, China. ⁶Department of Nematology, University of California, Riverside, CA 92521, USA. ⁷Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsin-Chu 300, Taiwan.

*These authors contributed equally to this work.

†Present address: College of plant protection, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China.

‡Corresponding author. E-mail: hailingj@ucr.edu



Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways

Arne Weiberg *et al.*

Science **342**, 118 (2013);

DOI: 10.1126/science.1239705

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/118.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.118.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/118.full.html#related>

This article **cites 31 articles**, 8 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/118.full.html#ref-list-1>

This article has been **cited by 1** articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/342/6154/118.full.html#related-urls>

16. S. Isayenkov, F. J. Maathuis, *Plant Signal. Behav.* **8**, e24665 (2013).
17. M. Zanetti *et al.*, *PLOS ONE* **5**, e10118 (2010).
18. S. de Bianchi *et al.*, *Plant Cell* **23**, 2659–2679 (2011).
19. B. Bailleul, P. Cardol, C. Breyton, G. Finazzi, *Photosynth. Res.* **106**, 179–189 (2010).
20. J. A. Cruz, C. A. Sacksteder, A. Kanazawa, D. M. Kramer, *Biochemistry* **40**, 1226–1237 (2001).
21. S. Eberhard, G. Finazzi, F. A. Wollman, *Annu. Rev. Genet.* **42**, 463–515 (2008).
22. N. E. Ioannidis, J. A. Cruz, K. Kotzabasis, D. M. Kramer, *PLOS ONE* **7**, e29864 (2012).
23. T. J. Avenso, J. A. Cruz, D. M. Kramer, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 5530–5535 (2004).
24. N. V. Paramonova, N. I. Shevyakova, V. V. Kuznetsov, *Russ. J. Plant Physiol.* **51**, 86–96 (2004).
25. E. Rintamäki, P. Martinsuo, S. Pursiheimo, E. M. Aro, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11644–11649 (2000).
26. G. Schönknecht, R. Hedrich, W. Junge, K. Raschke, *Nature* **336**, 589–592 (1988).
27. M. Tester, M. R. Blatt, *Plant Physiol.* **91**, 249–252 (1989).
28. K. Maxwell, G. N. Johnson, *J. Exp. Bot.* **51**, 659–668 (2000).

Acknowledgments: The authors are grateful to M. Zoratti and N. La Rascio for useful discussion and to J. Barber, N. Uozumi, P. Joliot, and N. Rolland for critical reading of the manuscript. We thank A. Spena for the pBIN RoC plasmid, L. Leanza for help with antibody production, and M. Simonetti for help with artwork. We thank Italy's PRIN (Research Projects of National Interest) (2010CSJX4F_005 to I.S.) and European Molecular Biology Organization Young Investigator Program for financial support (to I.S.) and the Aldo Gini Program for a short-term grant to V.C. to work in the laboratory of G.F.; the Ph.D. fellowship for L.C. was provided by the Italian Ministry of Education (L170). M.T. was supported by the French National Agency (ANR-2010-GENOM-BTV-002-01 Chloro-Type). G.F. was funded by the French National Agency "Project PhytAdapt" (ANR-NT09_567009), the Marie Curie Initial Training Network "AccliPhot" (FP7-PEOPLE-2012-ITN, grant agreement number 316427), and the Labex GRAL (Grenoble Alliance for Integrated Structural Cell Biology) grants. Author contributions: expression and purification of ATPK3: L.C.

and E.T.; electrophysiology: L.C., E.T., and I.S.; localization: L.C., E.F., and M.T.; silencing: L.C. and E.F.; pigment analysis: L.C. and T.M.; analysis of visible phenotype: L.C., T.M., I.S., and G.F.; electron microscopy and transmission electron microscopy service: L.C.; pmf measurements: M.T., V.C., and G.F. The study was designed by I.S., G.F., and G.M.G.; the paper was written by I.S. and G.F. This paper is dedicated to G. Franchin and E. Vaccher at Centro di Riferimento Oncologico (CRO), Aviano. Authors declare no conflict of interest. Additional data are in the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6154/114/suppl/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 and S2
References (29–42)

18 June 2013; accepted 27 August 2013
Published online 5 September 2013;
10.1126/science.1242113

Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways

Arne Weiberg,^{1,2,3*} Ming Wang,^{1,2,3*} Feng-Mao Lin,⁴ Hongwei Zhao,^{1,2,3†} Zhihong Zhang,^{1,2,3,5} Isgouhi Kaloshian,^{2,3,6} Hsien-Da Huang,^{4,7} Hailing Jin^{1,2,3‡}

Botrytis cinerea, the causative agent of gray mold disease, is an aggressive fungal pathogen that infects more than 200 plant species. Here, we show that some *B. cinerea* small RNAs (Bc-sRNAs) can silence *Arabidopsis* and tomato genes involved in immunity. These Bc-sRNAs hijack the host RNA interference (RNAi) machinery by binding to *Arabidopsis* Argonaute 1 (AGO1) and selectively silencing host immunity genes. The *Arabidopsis ago1* mutant exhibits reduced susceptibility to *B. cinerea*, and the *B. cinerea dcl1 dcl2* double mutant that can no longer produce these Bc-sRNAs displays reduced pathogenicity on *Arabidopsis* and tomato. Thus, this fungal pathogen transfers "virulent" sRNA effectors into host plant cells to suppress host immunity and achieve infection, which demonstrates a naturally occurring cross-kingdom RNAi as an advanced virulence mechanism.

Botrytis cinerea is a fungal pathogen that infects almost all vegetable and fruit crops and annually causes \$10 billion to \$100 billion in losses worldwide. With its broad host range, *B. cinerea* is a useful model for studying the pathogenicity of aggressive fungal pathogens. Many pathogens of plants and animals deliver effectors into host cells to suppress host immunity (1–4). All the pathogen effectors studied so

far are proteins. We found that small RNA (sRNA) molecules derived from *B. cinerea* can act as effectors to suppress host immunity.

sRNAs induce gene silencing by binding to Argonaute (AGO) proteins and directing the RNA-induced silencing complex (RISC) to genes with complementary sequences. sRNAs from both plant and animal hosts have been recognized as regulators in host-microbial interaction (5–8). Although sRNAs are also present in various fungi and oomycetes, including many pathogens (9–14), it has not been clear whether they regulate host-pathogen interaction.

To explore the role of *B. cinerea* sRNAs in pathogenicity, we profiled sRNA libraries prepared from *B. cinerea* (strain B05.10)–infected *Arabidopsis thaliana* Col-0 leaves collected at 0, 24, 48, and 72 hours after inoculation and from *B. cinerea*–infected *Solanum lycopersicum* (tomato) leaves and fruits at 0, 24, and 72 hours after inoculation. sRNA libraries prepared from *B. cinerea* mycelia, conidiospores, and total biomass after 10 days of culture were used as controls. By using

100 normalized reads per million *B. cinerea* sRNA reads as a cutoff, we identified a total of 832 sRNAs that were present in both *B. cinerea*–infected *Arabidopsis* and *S. lycopersicum* libraries and had more reads in these libraries than in the cultured *B. cinerea* libraries, with sequences exactly matching the *B. cinerea* B05.10 genome (15) but not *Arabidopsis* or *S. lycopersicum* genomes or cDNA (tables S1 to S3). The closest sequence matches in *Arabidopsis* or *S. lycopersicum* contained a minimum of two mismatches. Among them, 27 had predicted microRNA (miRNA)–like precursor structures. A similar number of miRNA-like sRNAs were found in *Sclerotinia sclerotiorum* (9). We found that 73 Bc-sRNAs could target host genes in both *Arabidopsis* and *S. lycopersicum* under stringent target prediction criteria (tables S3). Among them, 52 were derived from six retrotransposon long terminal repeats (LTR) loci in the *B. cinerea* genome, 13 were from intergenic regions of 10 loci, and eight were mapped to five protein-coding genes.

Some of the predicted plant targets, such as mitogen-activated protein kinases (MAPKs), are likely to function in plant immunity. To test whether Bc-sRNAs could indeed suppress host genes during infection, three Bc-sRNAs (Bc-siR3.1, Bc-siR3.2, and Bc-siR5) were selected for further characterization (table S2). These Bc-sRNAs were among the most abundant sRNAs that were 21 nucleotides (nt) in length and had potential targets likely to be involved in plant immunity in both *Arabidopsis* and *S. lycopersicum*. These sRNAs were also enriched after infection (Fig. 1, A and B; fig. S1; and table S2) and were the major sRNA products from their encoding loci, LTR retrotransposons (fig. S1). Bc-siR3.1 and Bc-siR3.2 were derived from the same locus with a 4-nt shift in sequence.

To determine whether Bc-sRNAs could trigger silencing of host genes, we examined the transcript levels of the predicted target genes after *B. cinerea* infection. The following *Arabidopsis* genes were targeted in the coding regions and were suppressed after *B. cinerea* infection: *mitogen activated protein kinase 2* (MPK2) and *MPK1*, which are targeted

¹Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. ²Center for Plant Cell Biology, University of California, Riverside, CA 92521, USA. ³Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA. ⁴Department of Biological Science and Technology, National Chiao Tung University, Hsin-Chu 300, Taiwan. ⁵College of Horticulture, Shenyang Agricultural University, Shenyang, Liaoning 110866, China. ⁶Department of Nematology, University of California, Riverside, CA 92521, USA. ⁷Institute of Bioinformatics and Systems Biology, National Chiao Tung University, Hsin-Chu 300, Taiwan.

*These authors contributed equally to this work.

†Present address: College of plant protection, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China.

‡Corresponding author. E-mail: hailingj@ucr.edu

by Bc-siR3.2; an oxidative stress-related gene, *peroxiredoxin* (*PRXIII*), which is targeted by Bc-siR3.1; and *cell wall-associated kinase* (*WAK*), which is targeted by Bc-siR5 (Fig. 1C). In contrast, the plant defense marker genes *PDF1.2* and *BIK1* (16), which do not contain the Bc-sRNA target sites, were highly induced upon *B. cinerea* infection (Fig. 1C). We conclude that suppression of some but not all genes is a result of sequence-specific sRNA interaction and not due to cell death within infected lesions. Bc-siR3.2, which silences *Arabidopsis* *MPK1* and *MPK2*, was enriched also in *S. lycopersicum* leaves upon *B. cinerea* infection (Fig. 1B) and was predicted to target another member of the MAPK signaling cascade in *S. lycopersicum*, *MAPKKK4* (table S2). Expression of *MAPKKK4* was indeed suppressed upon *B. cinerea* infection (Fig. 1D).

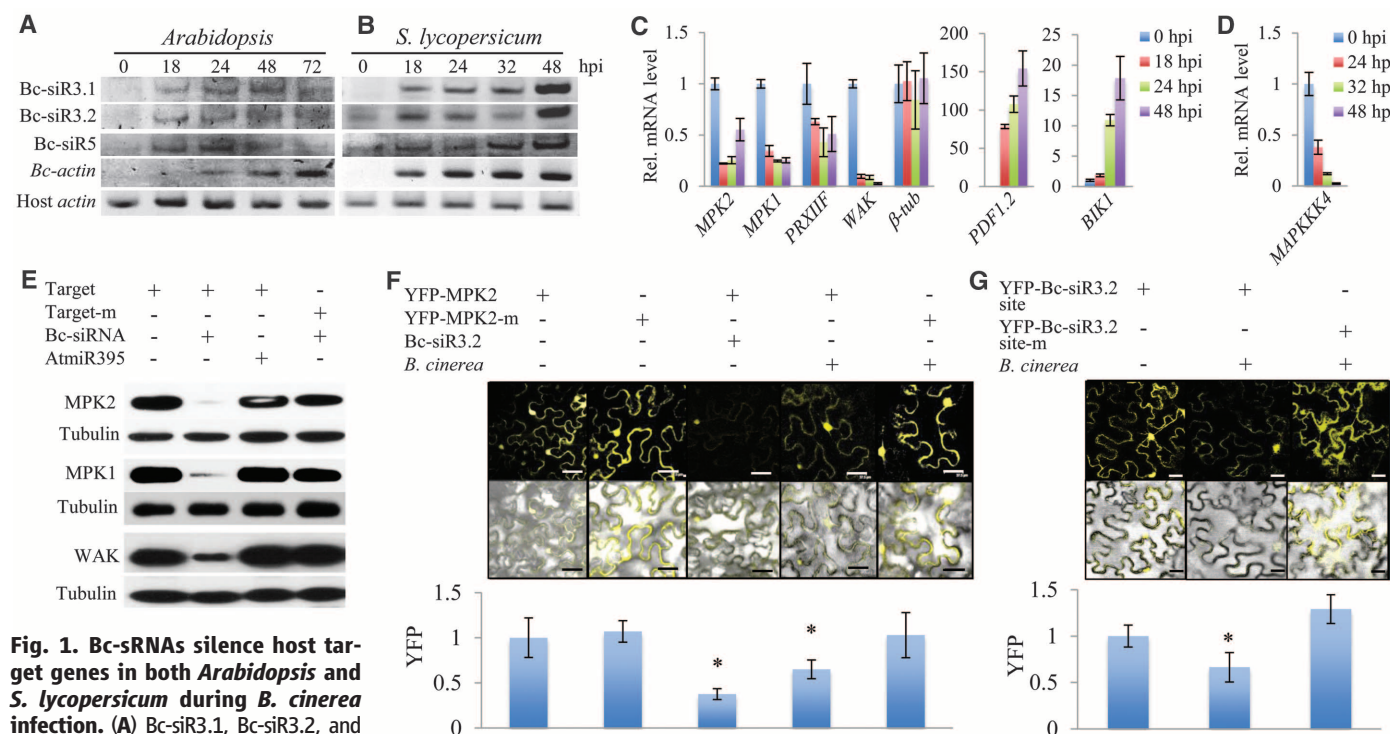
To confirm that the suppression of the targets was indeed triggered by Bc-sRNAs, we performed coexpression assays in *Nicotiana benthamiana*. Expression of hemagglutinin (HA)-epitope tagged *MPK2*, *MPK1*, and *WAK* was reduced when they were coexpressed with the corresponding Bc-sRNAs

but not when coexpressed with *Arabidopsis* miR395, which shared no sequence similarity (Fig. 1E). The silencing was abolished, however, when the target genes carried a synonymously mutated version of the relevant Bc-sRNA target sites (Fig. 1E and fig. S2A). We also observed suppression of yellow fluorescent protein (YFP)-tagged target *MPK2* by *B. cinerea* infection at 24 hours after inoculation (Fig. 1F and fig. S2B); when the Bc-siR3.2 target site of *MPK2* was mutated, infection by *B. cinerea* failed to suppress its expression (Fig. 1F and fig. S2B). Thus, Bc-siR3.2 delivered from *B. cinerea* is sufficient for inducing silencing of wild-type *MPK2* but cannot silence target site-mutated *MPK2*. Similarly, of the YFP-sensors with wild-type or mutated Bc-siR3.2 target sites (fig. S2C), only the wild-type sensor was suppressed after *B. cinerea* infection (Fig. 1G).

To test the effect of Bc-sRNAs on host plant immunity, we generated transgenic *Arabidopsis* plants that ectopically expressed Bc-siR3.1, Bc-siR3.2, or Bc-siR5 using a plant artificial miRNA vector (Fig. 2A) (17). These Bc-sRNA expression (Bc-sRNAox) lines showed normal morphology

and development without pathogen challenge when compared with the wild-type plants, and expression of the target genes was suppressed (Fig. 2B). With pathogen challenge, all of the Bc-sRNAox lines displayed enhanced susceptibility to *B. cinerea* (Fig. 2, C and E). The results indicate that these Bc-sRNAs play a positive role in *B. cinerea* pathogenicity.

Enhanced disease susceptibility of the Bc-sRNAox lines suggests that the target genes of these Bc-sRNAs are likely to be involved in host immunity against *B. cinerea*. Plants with mutated target genes showed normal morphology and development without pathogen challenge. The *Arabidopsis* targets of Bc-siR3.2, *MPK1* and *MPK2*, are homologs that share 87% amino acid identity. These genes are functionally redundant and are coactivated in response to various stress factors (18). The *mpk1 mpk2* double mutant exhibited enhanced susceptibility to *B. cinerea* (Fig. 2, D and E). A transferred-DNA knockout mutant of the Bc-siR5 target *WAK* (SALK_089827) (fig. S3A) also displayed enhanced susceptibility to *B. cinerea* (Fig. 2, D and E). Consistent



revealed target silencing by means of Western blot analysis. Coexpression of AtmiR395 or target site-mutated versions of target genes was used as controls. (F) Expression of YFP-*MPK2* or its synonymously mutated version (YFP-*MPK2*-m) after infection of *B. cinerea* was observed with confocal microscopy. Coexpression of YFP-*MPK2* and Bc-siR3.2 was used as a control. (G) Expression of the YFP sensors carrying a Bc-siR3.2 target site of *MPK2* or a Bc-siR3.2 target site-m was analyzed after infection of *B. cinerea*. Samples were examined at 24 hours after inoculation. (Top) YFP. (Bottom) YFP/bright field overlay. Scale bars [(F) and (G)], 37.5 μ m. Error bars indicate SD of 20 images [(F) and (G)]. The asterisk indicates significant difference (two-tail *t*-test; *P* < 0.01). Similar results were obtained in three biological replicates in (E) to (G).

revealed target silencing by means of Western blot analysis. Coexpression of AtmiR395 or target site-mutated versions of target genes was used as controls. (F) Expression of YFP-*MPK2* or its synonymously mutated version (YFP-*MPK2*-m) after infection of *B. cinerea* was observed with confocal microscopy. Coexpression of YFP-*MPK2* and Bc-siR3.2 was used as a control. (G) Expression of the YFP sensors carrying a Bc-siR3.2 target site of *MPK2* or a Bc-siR3.2 target site-m was analyzed after infection of *B. cinerea*. Samples were examined at 24 hours after inoculation. (Top) YFP. (Bottom) YFP/bright field overlay. Scale bars [(F) and (G)], 37.5 μ m. Error bars indicate SD of 20 images [(F) and (G)]. The asterisk indicates significant difference (two-tail *t*-test; *P* < 0.01). Similar results were obtained in three biological replicates in (E) to (G).

with this, Bc-sRNAox lines as well as *mpk1 mpk2* and *wak* showed lower induction of the defense marker gene *BIK1* (fig. S3B). These results suggest that the *MPK1*, *MPK2*, and *WAK* genes, all of which are targeted by Bc-sRNAs, participate in the plant's immune response to *B. cinerea*. To determine whether *MAPKKK4* is involved in *S. lycopersicum* defense response against *B. cinerea*, we applied the virus-induced gene silencing (VIGS) approach to knock down *MAPKKK4* in *S. lycopersicum* using tobacco rattle virus (TRV) (fig. S4A) (19). VIGS of TRV-*MAPKKK4* caused a dwarf phenotype (fig. S4B).

The *MAPKKK4*-silenced plants showed enhanced disease susceptibility in response to *B. cinerea* and contained >15 times more fungal biomass than that of the control plants (Fig. 2F). We conclude that Bc-sRNAs silence plant genes to suppress host immunity during early infection.

These fungal sRNAs hijack the plant's own gene silencing mechanism. Sixty-three of the 73 Bc-sRNAs that had predicted *Arabidopsis* and *S. lycopersicum* targets were 20 to 22 nt in length with a 5' terminal U (table S3). This sRNA structure is favored for binding to AGO1 in *Arabidopsis* (20, 21). In order to determine whether Bc-sRNAs

act through *Arabidopsis* AGO1, we immunoprecipitated AGO1 from *B. cinerea*-infected *Arabidopsis* collected at 24, 32, and 48 hours after inoculation and analyzed the AGO1-associated sRNAs. Bc-siR3.1, Bc-siR3.2, and Bc-siR5 were clearly detected in the AGO1-associated fraction pulled down from the infected plant samples but hardly in the control (Fig. 3A) or in the AGO2- and AGO4-associated sRNA fractions (fig. S5). The sRNAs that had no predicted plant targets or had predicted targets that were not down-regulated by *B. cinerea* infection were not found in the AGO1-associated fractions (fig. S6).

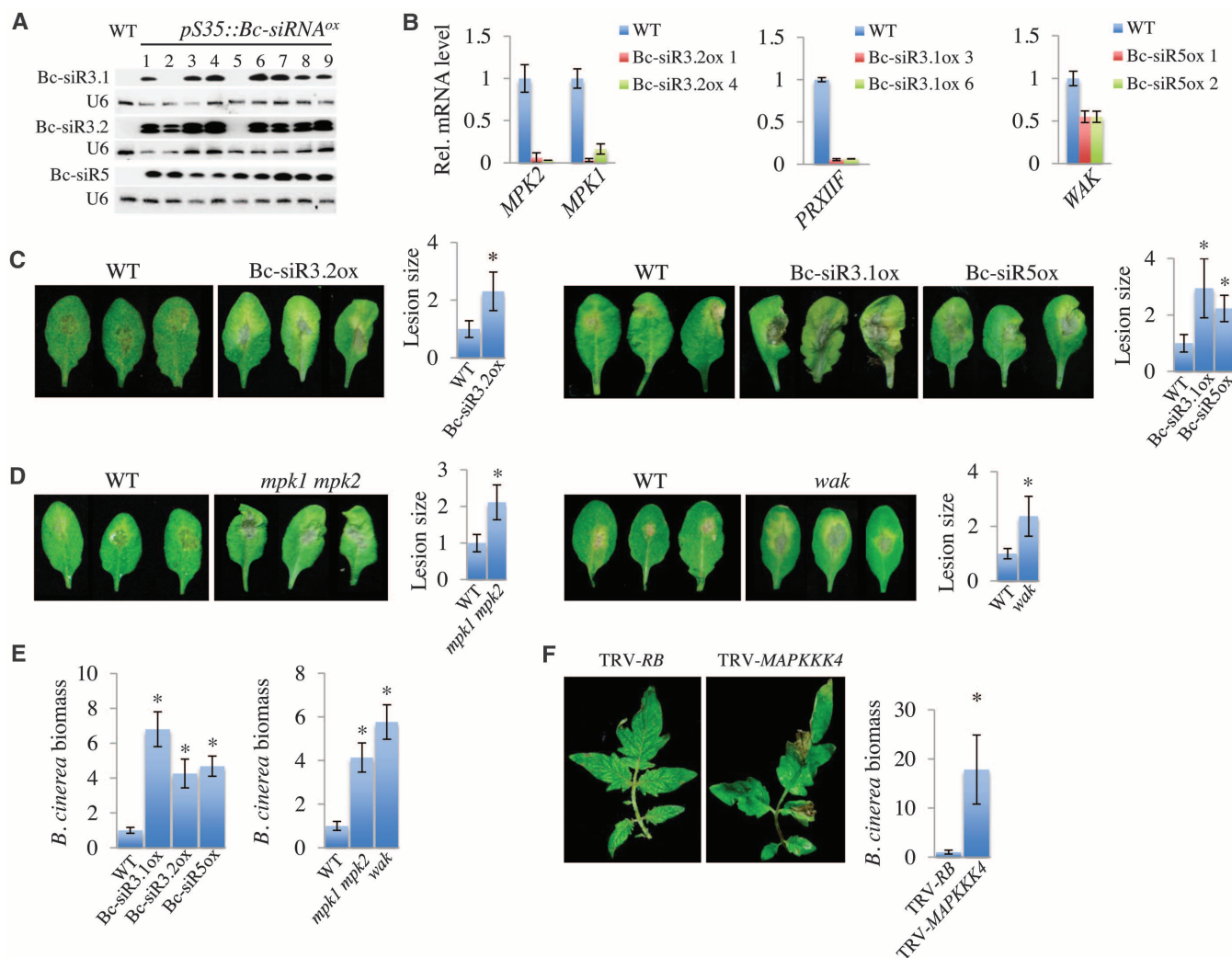


Fig. 2. Bc-sRNAs trigger silencing of host targets that are involved in host immunity. (A) Expression of Bc-siR3.1, Bc-siR3.2, or Bc-siR5 in transgenic *Arabidopsis* ectopically expressing Bc-sRNAs under the Cauliflower Mosaic Virus promoter 35S (Bc-sRNAox) was examined by means of Northern blot analysis. Highly expressed lines were selected for the following experiments. (B) Bc-sRNAox lines showed constitutive silencing of respective Bc-sRNA target genes measured with quantitative RT-PCR. Two independent lines for each Bc-sRNA were examined. Similar results were observed in two generations of the selected transgenic lines. (C) Bc-sRNAox plants exhibited enhanced disease susceptibility to *B. cinerea* as compared with wild type. (D) Loss-of-function mutants of Bc-siR3.2 and Bc-siR5 targets *mpk1 mpk2* and *wak* displayed enhanced disease susceptibility. In all pathogen assays [(C) and (D)], lesion sizes were measured at 96 hours after inoculation. Error bars indicate the SD of 20 leaves.

(E) Biomass of *B. cinerea* was measured with quantitative PCR at 96 hours after inoculation. Error bars indicate SD of three technical replicates. For (C), (D), and (E), similar results were obtained from three biological repeats. (F) VIGS of *MAPKKK4* exhibited enhanced disease susceptibility to *B. cinerea* in *S. lycopersicum* (examined at 72 hours after inoculation) as compared with control plants (TRV-RB). RB is a late-blight resistance gene that is not present in tomato. We chose to use a TRV vector with a fragment from a foreign gene as a control to eliminate the potential side effect of viral disease symptoms caused by TRV empty vector. Spray inoculation was used because silencing sectors are not uniform within the VIGS plants. Three sets of experiments with each of 6 to 10 plants for each construct were performed, and similar results were obtained. The asterisk indicates significant difference (two-tail *t*-test, $P < 0.01$) in (C) to (F).

If AGO1 plays an essential role in Bc-sRNA-mediated host gene silencing, we would expect to see reduced disease susceptibility in the *ago1* mutant because these Bc-sRNAs could no longer suppress host immunity genes. For plants carrying the *ago1-27* mutant allele (22) and were inoculated with *B. cinerea*, the disease level was significantly less than on the wild type (Fig. 3B and fig. S7A). Consistent with this, *BIK1* induction was increased compared with that of the wild-type (fig. S7B). Furthermore, the expression of Bc-siR3.2 targets *MPK2* and *MPK1*, Bc-siR3.1 target *PRXIIIF*, and Bc-siR5 target *WAK* in *ago1-27* was not suppressed compared with those in wild-type infected plants after *B. cinerea* infection (Fig. 3C). On the contrary, *Arabidopsis* miRNA biogenesis mutant *dicer-like* (*dcl*) 1-7 that shows similar morphological defects to *ago1-27* exhibited an enhanced disease level to *B. cinerea* (Fig. 3D). These results suggest that the increased resistance phenotype we observed in *ago1-27* is not caused by any reduced vigour or pleiotropic phenotype but was due to the function of the Bc-sRNAs, and that *Arabidopsis* DCL1 is not required for the function of Bc-sRNAs.

Thus, Bc-sRNAs evidently hijacked host RNAi machinery by loading into AGO1; the complex in turn suppressed host immunity genes.

To delete the siR3 and siR5 loci from the *B. cinerea* genome by homologous recombination would be an ideal way to confirm their function; however, it is not feasible because siR3 is from a LTR with three copies and siR5 is from a LTR with 13 copies. To better understand the function and biogenesis of the Bc-sRNAs, we chose to knock out the *B. cinerea* DCL genes, which encode the core sRNA processing enzymes. *B. cinerea* strain B05.10 possesses two Dicer-like genes (*Bc-DCL1* and *Bc-DCL2*) (fig. S8). We generated *dcl1* and *dcl2* single and *dcl1 dcl2* double knockout mutant strains through homologous recombination (fig. S9, A and B). We found that *dcl1* and *dcl2* single mutants showed reduced growth and delayed sporulation (fig. S9C). The *dcl1 dcl2* double mutant displayed a more obvious phenotype than that of each of the single mutants, suggesting partial functional redundancy between DCL1 and DCL2 in *B. cinerea*. Bc-siR3.1, Bc-siR3.2, and Bc-siR5 could not be detected in the *dcl1 dcl2*

double mutant (Fig. 4A), indicating that they were DCL-dependent, whereas two other Bc-sRNAs, Bc-miR2 and Bc-siR1498, could still be detected in *dcl1 dcl2* double mutant (fig. S9D). Fungi have diverse sRNA biogenesis pathways, and not all sRNAs are DCL-dependent (12). The *dcl1 dcl2* double mutant caused significantly smaller lesions than those of the wild type or *dcl1* and *dcl2* single mutants on both *Arabidopsis* and *S. lycopersicum* leaves (Fig. 4, B and C), in consistence with the significantly reduced fungal biomass at 72 hours after inoculation in *Arabidopsis* and 48 hours after inoculation in *S. lycopersicum* (fig. S10), which indicates that the virulence of the *dcl1 dcl2* mutant was greatly reduced. These results further support the conclusion that Bc-sRNAs—particularly Bc-siR3.1, Bc-siR3.2, and Bc-siR5, which depend on *B. cinerea* DCL function—contribute to the pathogenicity of *B. cinerea*. Mutation of *dcl1* or *dcl2* in *B. cinerea* caused delayed growth and sporulation (fig. S9C) but had no effect on pathogenicity (Fig. 4, B and C). Furthermore, expression of the YFP sensor carrying the Bc-siR3.2 target site in *N. benthamiana* was silenced when infected

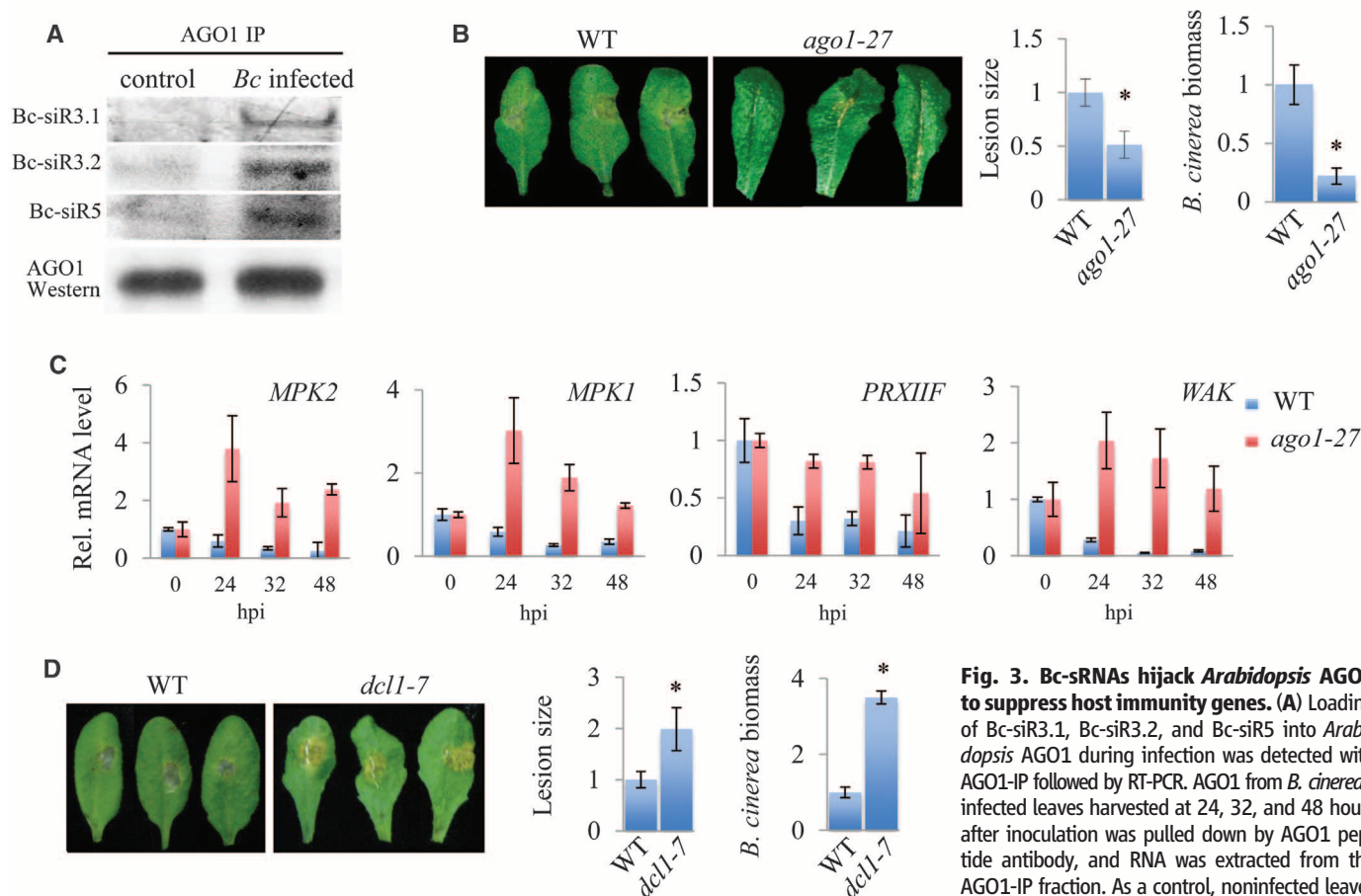


Fig. 3. Bc-sRNAs hijack *Arabidopsis* AGO1 to suppress host immunity genes. (A) Loading of Bc-siR3.1, Bc-siR3.2, and Bc-siR5 into *Arabidopsis* AGO1 during infection was detected with AGO1-IP followed by RT-PCR. AGO1 from *B. cinerea*-infected leaves harvested at 24, 32, and 48 hours after inoculation was pulled down by AGO1 peptide antibody, and RNA was extracted from the AGO1-IP fraction. As a control, noninfected leaves mixed with *B. cinerea* mycelium (at least twice as

much as that in *B. cinerea*-infected leaves at 48 hours after inoculation) were used to rule out any binding between AGO1 and Bc-sRNAs during the experimental procedures. Similar results were obtained from at least three biological repeats. (B) *Arabidopsis ago1-27* exhibited reduced disease susceptibility to *B. cinerea* as compared with the wild type. Lesion size of at least 20 leaves and fungal biomass were measured at 96 hours after inoculation. (C) Silencing of *MPK2*, *MPK1*, *PRXIIIF*, and *WAK* during *B. cinerea* infection was abolished in *ago1-27*. (D) *Arabidopsis dcl1-7* exhibited enhanced disease susceptibility to *B. cinerea* as compared with the wild type. Similar results were obtained from three biological repeats [(B) to (D)]. The asterisk indicates significant difference (two-tail *t*-test, $P < 0.01$) in (B) and (D).

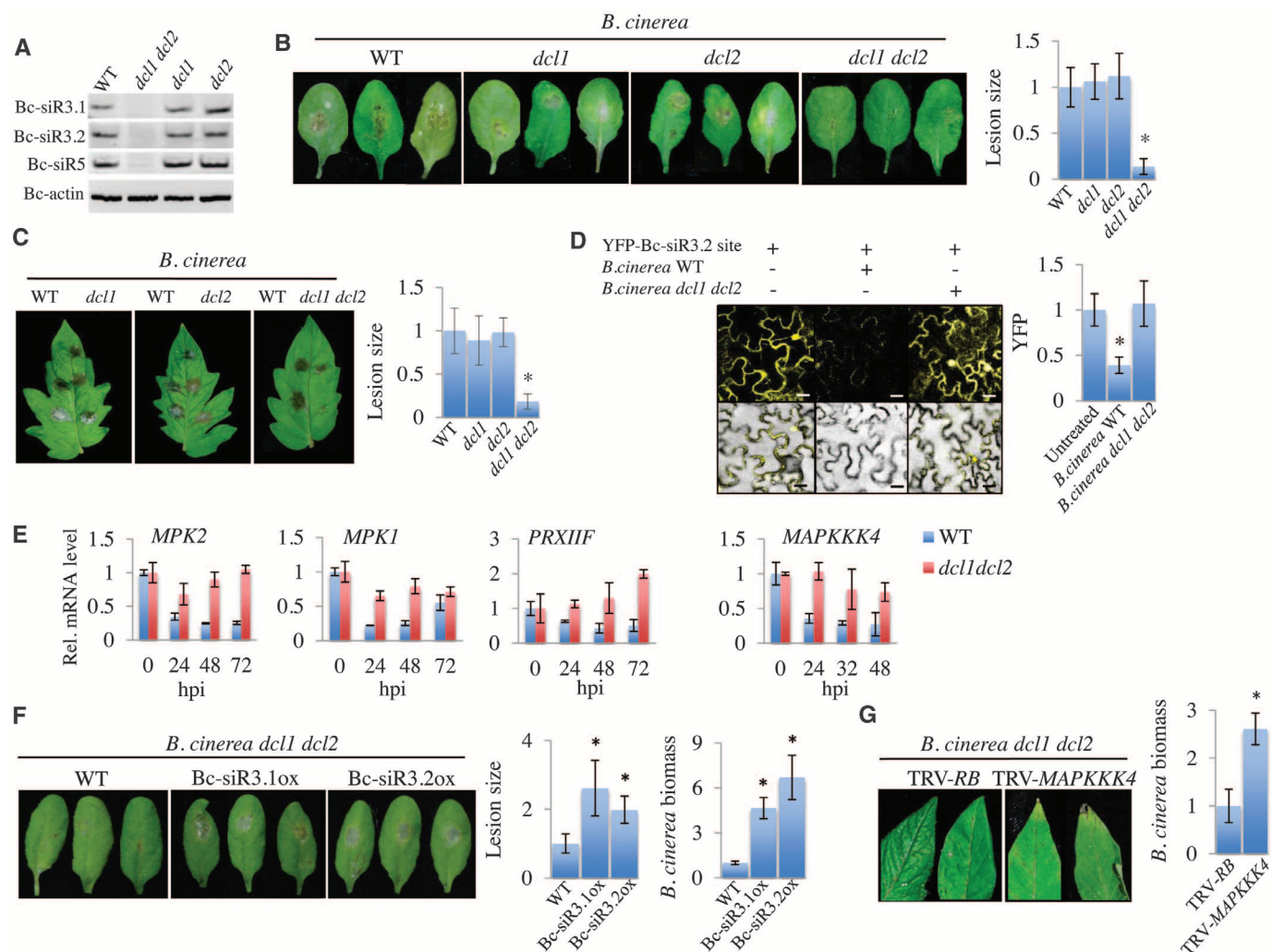


Fig. 4. *B. cinerea* dcl1 dcl2 double mutant is compromised in virulence.

(A) *B. cinerea* dcl1 dcl2 double mutant, but not dcl1 or dcl2 single mutants, was impaired in generating Bc-siR3.1, Bc-siR3.2, and Bc-siR5 as revealed with RT-PCR. *B. cinerea* dcl1 dcl2 double mutant, but not dcl1 or dcl2 single mutants, produced much weaker disease symptoms than did the wild type in (B) *Arabidopsis* and (C) *S. lycopersicum*, as demonstrated by the lesion size measured of 20 leaves at 96 and 48 hours after inoculation, respectively. Similar results were obtained from three biological repeats. (D) Expression of the sensor YFP-Bc-siR3.2 target site was silenced by wild-type *B. cinerea* upon infection, but not by the dcl1 dcl2 mutant at 24 hours after inoculation. Scale

bar, 75 μ m. Error bars indicate SD of 20 images. Experiments were repeated two times with similar results. (E) *B. cinerea* dcl1 dcl2 mutant was compromised in suppression of *MPK2*, *MPK1*, and *PRXIIIF* in *Arabidopsis* and *MAPKKK4* in *S. lycopersicum*. Similar results were seen in two biological repeats. (F) *Arabidopsis* Bc-siR3.1ox and Bc-siR3.2ox lines were more susceptible to *B. cinerea* dcl1 dcl2 strain than was Col-0 wild type. (G) Enhanced disease phenotype of dcl1 dcl2 infection was also observed on TRV-MAPKKK4-silenced *S. lycopersicum* plants. Experiments in (F) and (G) were repeated three times with similar results. *B. cinerea* biomass was quantified at 96 hours after inoculation. The asterisk [in (B), (C), (D), (F), and (G)] indicates significant difference (two-tail *t*-test; *P* < 0.01).

with wild-type *B. cinerea*. The suppression was abolished when inoculated with the dcl1 dcl2 strain (Fig. 4D), indicating that the dcl1 dcl2 double mutant was unable to generate Bc-siR3.2 to suppress the target. We also confirmed the inability of dcl1 dcl2 to suppress Bc-siR3.1 and Bc-siR3.2 target genes *MPK2*, *MPK1*, and *PRXIIIF* in *Arabidopsis* and *MAPKKK4* in tomato upon infection (Fig. 4E). Consistent with this, the dcl1 dcl2 virulence was partially restored when infected on *Arabidopsis* Bc-siR3.1ox and Bc-siR3.2ox plants as well as in tomato TRV-MAPKKK4-silenced plants (Fig. 4, F and G).

Animal and plant pathogens have evolved virulence or effector proteins to counteract host immune responses. Various protein effectors have

been predicted or discovered in fungal or oomycete pathogens from whole-genome sequencing and secretome analysis (2, 3), although delivery mechanisms are still under active investigation (23–27). Here, we show that sRNAs as well can act as effectors through a mechanism that silences host genes in order to debilitate plant immunity and achieve infection. The sRNAs from *B. cinerea* hijack the plant RNAi machinery by binding to AGO proteins, which in turn direct host gene silencing. Another fungal plant pathogen, *Verticillium dahliae*, also depends on AGO1 function for its pathogenicity (28). The implications of these findings may extend beyond plant gray mold disease caused by *B. cinerea* and suggest an extra mechanism underlying pathogenesis promoted by sophisticated

pathogens with the capability to generate and deliver small regulatory RNAs into hosts to suppress host immunity.

References and Notes

- H. Ashida et al., *Curr. Opin. Microbiol.* **14**, 16–23 (2011).
- M. Rafiqi, J. G. Ellis, V. A. Ludowici, A. R. Hardham, P. N. Dodds, *Curr. Opin. Plant Biol.* **15**, 477–482 (2012).
- T. O. Bozkurt, S. Schornack, M. J. Banfield, S. Kamoun, *Curr. Opin. Plant Biol.* **15**, 483–492 (2012).
- H. Hilbi, A. Haas, *Traffic* **13**, 1187–1197 (2012).
- S. Katiyar-Agarwal, H. Jin, *Annu. Rev. Phytopathol.* **48**, 225–246 (2010).
- V. Ruiz-Ferrer, O. Voinnet, *Annu. Rev. Plant Biol.* **60**, 485–510 (2009).
- B. Wessner, L. Gryadunov-Masutti, H. Tschann, N. Bachl, E. Roth, *Exerc. Immunol. Rev.* **16**, 22–39 (2010).

8. X. Zhang *et al.*, *Mol. Cell* **42**, 356–366 (2011).
9. J. Zhou *et al.*, *Mol. Gen. Genet.* **287**, 275–282 (2012).
10. D. Qutob, B. Patrick Chapman, M. Gijzen, *Nature Comm.* **4**, 1349 (2013).
11. N. Jiang, Y. Yang, G. Janbon, J. Pan, X. Zhu, *PLoS ONE* **7**, e52734 (2012).
12. H. C. Lee *et al.*, *Mol. Cell* **38**, 803–814 (2010).
13. C. C. Nunes *et al.*, *BMC Genomics* **12**, 288 (2011).
14. V. Raman *et al.*, *BMC Genomics* **14**, 326 (2013).
15. J. Amselem *et al.*, *PLOS Genet.* **7**, e1002230 (2011).
16. P. Veronese *et al.*, *Plant Cell* **18**, 257–273 (2006).
17. R. Schwab, S. Ossowski, M. Riester, N. Warthmann, D. Weigel, *Plant Cell* **18**, 1121–1133 (2006).
18. D. Ortiz-Masia, M. A. Perez-Amador, J. Carbonell, M. J. Marcote, *FEBS Lett.* **581**, 1834–1840 (2007).
19. Y. L. Liu, M. Schiff, S. P. Dinesh-Kumar, *Plant J.* **31**, 777–786 (2002).
20. S. J. Mi *et al.*, *Cell* **133**, 116–127 (2008).
21. T. A. Montgomery *et al.*, *Cell* **133**, 128–141 (2008).
22. J. B. Morel *et al.*, *Plant Cell* **14**, 629–639 (2002).
23. S. D. Kale *et al.*, *Cell* **142**, 284–295 (2010).
24. S. Wawra *et al.*, *Curr. Opin. Microbiol.* **15**, 685–691 (2012).
25. M. Rafiqi *et al.*, *Plant Cell* **22**, 2017–2032 (2010).
26. S. Schornack *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17421–17426 (2010).
27. S. Wawra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 2096–2101 (2012).
28. U. Ellendorff, E. F. Fradin, R. de Jonge, B. P. Thomma, *J. Exp. Bot.* **60**, 591–602 (2009).

Acknowledgments: We thank Y. Qi for AGO1 antibody; J. Carrington for the AGO1 and AGO2 tagged lines; H. Vaucheret for *ago1-27* mutant; M. J. Marcote for *mpk1 mpk2* seeds; and P. Karlovsky for pPK2 binary vector and

A. tumefaciens strain AGL1 for *B. cinerea* transformation and gene knockout. This work was supported by an NIH grant (R01 GM093008), an NSF Career Award (MCB-0642843), an NSF award (IOS-1257576), and an AES-CE award (PPA-7517H) to H.J.; National Science Council of the Republic of China (NSC 101-2311-B-009-003-MY3 and NSC 100-2627-B-009-002) and UST-UCSD I-RiCE Program (NSC 101-2911-I-009-101) grants to H.-D.H.

Supplementary Materials

www.sciencemag.org/content/342/6154/118/suppl/DC1
Materials and Methods
Fig. S1 to S10
Tables S1 to S4
References (29–32)

26 April 2013; accepted 19 August 2013
10.1126/science.1239705

Crystal Structure of Na⁺, K⁺-ATPase in the Na⁺-Bound State

Maria Nyblom,^{1,2,*†} Hanne Poulsen,^{1,2,3,*‡} Pontus Gourdon,^{1,2,3,*} Linda Reinhard,^{1,2,§} Magnus Andersson,⁴ Erik Lindahl,^{4,5} Natalya Fedosova,^{1,6} Poul Nissen^{1,2,3,†}

The Na⁺, K⁺-adenosine triphosphatase (ATPase) maintains the electrochemical gradients of Na⁺ and K⁺ across the plasma membrane—a prerequisite for electrical excitability and secondary transport. Hitherto, structural information has been limited to K⁺-bound or ouabain-blocked forms. We present the crystal structure of a Na⁺-bound Na⁺, K⁺-ATPase as determined at 4.3 Å resolution. Compared with the K⁺-bound form, large conformational changes are observed in the α subunit whereas the β and γ subunit structures are maintained. The locations of the three Na⁺ sites are indicated with the unique site III at the recently suggested IIIb, as further supported by electrophysiological studies on leak currents. Extracellular release of the third Na⁺ from IIIb through IIIa, followed by exchange of Na⁺ for K⁺ at sites I and II, is suggested.

The Na⁺, K⁺-adenosine triphosphatase (ATPase) is typically a ternary complex of a large catalytic α subunit associated with two smaller subunits, β and γ (Fig. 1A). Different isoforms combine to form kinetically distinct complexes in different cells and tissues (*1*). During the ATP-driven transport cycle, three cytoplasmic Na⁺ are exported in exchange for two extracellular K⁺ through alternating E1/E1P and E2P/E2 states (Fig. 1B), where E1 and E2 denote high affinity for

sodium and potassium ions, respectively, and P is phosphorylated. Intracellular Na⁺ and ATP binding stimulate phosphorylation of a conserved aspartic acid residue (Asp³⁶⁹ in pig α₁ isoform; see alignment in fig. S1), forming the sodium-occluded [Na₃]E1P-adenosine diphosphate (ADP) state. Conformational changes and ADP release open an extracellular pathway in the E2P state, and Na⁺ release and K⁺ binding stimulate dephosphorylation, yielding the potassium-occluded [K₂]E2P_i and [K₂]E2 states. Subsequent ATP binding and cytoplasmic K⁺ release lead to the sodium-bound E1 states.

Previously, K⁺-bound crystal structures representing the occluded E2P_i state as mimicked by magnesium fluoride ([K₂]E2-MgF_x) (2, 3) and structures with the inhibitory steroid ouabain (low-affinity [K₂]E2-MgF_x and high-affinity [Mg]E2P) have been elucidated (4, 5). The intracellular C-terminal tail of the α subunit plays an important role in Na⁺ binding (2, 6–10), apparently by controlling an ion pathway (6, 7), through a mechanism affected by many of the α₂ and α₃ isoform mutations that are associated with neurological diseases (11, 12). However, further elucidation of the transport mechanism of the Na⁺, K⁺-ATPase has been limited by the lack of a structure of the Na⁺-bound state, and in particular the location of the third Na⁺ site has remained unsettled.

We present here the crystal structure determined at 4.3 Å resolution of the Na⁺, K⁺-ATPase in the [Na₃]E1P-ADP state (pig renal α₁β₁γ enzyme) as mimicked by an ADP-AlF₄[−] complex (materials and methods and table S1) for which Na⁺ saturation was further stabilized by the presence of oligomycin. The structure was determined from an unbiased electron density map derived by single isomorphous replacement with anomalous scattering (SIRAS), using hexatantalum dodecaboride (Ta₆Br₁₂) derivatized crystals, followed by density modification procedures (Fig. 1C and fig. S2). Model building using sharpened maps and restrained refinement produced a final model with *R*_{work} and *R*_{free} of 26.1 and 28.8%, respectively. The structure represents two nearly identical complexes in the asymmetric unit (chains A-B-G and C-D-E) and displays bilayer features in the electron density (figs. S2 to S4).

The ability of the E1-AlF₄[−]-ADP complex to occlude three Na⁺ under crystallization-like conditions was confirmed by time-course measurements of ²²Na⁺ deocclusion at 0°C (Fig. 1F). The monoexponential fit resulted in the maximal number of 2.5 nmol of Na⁺ per nmol of ADP binding sites (i.e., 83% occupancy) at 1 mM ²²Na⁺ and the deocclusion rate constant of 0.02 s^{−1}. Assuming a Hill coefficient of 3 for the cooperative Na⁺ binding, the ion concentration required for the half-maximal saturation of the sites (*K*_{0.5} for Na⁺) was calculated to be 0.58 mM, consistent with previous findings (13). Thus, Na⁺ concentration under crystallization conditions (>80 mM) was more than two orders of magnitude higher than the *K*_{0.5}, enough to saturate all three sites.

The α subunit represents a Na⁺-occluded form of the transmembrane (TM) domain, with the cytoplasmic A, P, and N domains arranged for phosphorylation (Fig. 1, C and D) as observed for sarcoplasmic reticulum Ca²⁺ ATPase 1a (SERCA1a) in the equivalent, Ca²⁺-occluded state (fig. S4B) (14–16). Thus, compared with K⁺-bound forms, the α subunit is characterized by a different organization of the TM helices and a compact configuration of the cytoplasmic domains activating the phosphorylation site (Fig. 1, C and D, and fig. S5). Relative to the P domain, the A domain has undergone a rigid-body rota-

¹Centre for Membrane Pumps in Cells and Disease—PUMPKin, Danish National Research Foundation, DK-8000 Aarhus, Denmark.

²Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, DK-8000 Aarhus, Denmark. ³Danish Research Institute for Translational Neuroscience—DANDRITE, Nordic-EMBL Partnership of Molecular Medicine, Aarhus, Denmark.

⁴Science for Life Laboratory, Theoretical and Computational Biophysics, Department of Theoretical Physics, Swedish e-Science Research Center, KTH Royal Institute of Technology, SE-171 21 Solna, Sweden. ⁵Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden. ⁶Department of Biomedicine, Aarhus University, Ole Worms Allé 4, Building 1182, DK-8000 Aarhus, Denmark.

*These authors contributed equally to this work.

†Present address: Novo Nordisk A/S, Novo Nordisk Park, B9.2.31, DK-2760 Maalov, Denmark.

‡Corresponding author. E-mail: hp@mb.au.dk (H.P.); pn@mb.au.dk (P.N.)

§Present address: Department of Cell and Molecular Biology, Karolinska Institutet, Box 285, SE-171 77 Stockholm, Sweden.



Crystal Structure of Na⁺, K⁺-ATPase in the Na⁺-Bound State

Maria Nyblom *et al.*

Science **342**, 123 (2013);

DOI: 10.1126/science.1243352

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 4, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/123.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/09/18/science.1243352.DC1.html>

This article **cites 58 articles**, 11 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/123.full.html#ref-list-1>

8. X. Zhang *et al.*, *Mol. Cell* **42**, 356–366 (2011).
9. J. Zhou *et al.*, *Mol. Gen. Genet.* **287**, 275–282 (2012).
10. D. Qutob, B. Patrick Chapman, M. Gijzen, *Nature Comm.* **4**, 1349 (2013).
11. N. Jiang, Y. Yang, G. Janbon, J. Pan, X. Zhu, *PLoS ONE* **7**, e52734 (2012).
12. H. C. Lee *et al.*, *Mol. Cell* **38**, 803–814 (2010).
13. C. C. Nunes *et al.*, *BMC Genomics* **12**, 288 (2011).
14. V. Raman *et al.*, *BMC Genomics* **14**, 326 (2013).
15. J. Amselem *et al.*, *PLOS Genet.* **7**, e1002230 (2011).
16. P. Veronese *et al.*, *Plant Cell* **18**, 257–273 (2006).
17. R. Schwab, S. Ossowski, M. Riester, N. Warthmann, D. Weigel, *Plant Cell* **18**, 1121–1133 (2006).
18. D. Ortiz-Masia, M. A. Perez-Amador, J. Carbonell, M. J. Marcote, *FEBS Lett.* **581**, 1834–1840 (2007).
19. Y. L. Liu, M. Schiff, S. P. Dinesh-Kumar, *Plant J.* **31**, 777–786 (2002).
20. S. J. Mi *et al.*, *Cell* **133**, 116–127 (2008).
21. T. A. Montgomery *et al.*, *Cell* **133**, 128–141 (2008).
22. J. B. Morel *et al.*, *Plant Cell* **14**, 629–639 (2002).
23. S. D. Kale *et al.*, *Cell* **142**, 284–295 (2010).
24. S. Wawra *et al.*, *Curr. Opin. Microbiol.* **15**, 685–691 (2012).
25. M. Rafiqi *et al.*, *Plant Cell* **22**, 2017–2032 (2010).
26. S. Schornack *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17421–17426 (2010).
27. S. Wawra *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 2096–2101 (2012).
28. U. Ellendorff, E. F. Fradin, R. de Jonge, B. P. Thomma, *J. Exp. Bot.* **60**, 591–602 (2009).

Acknowledgments: We thank Y. Qi for AGO1 antibody; J. Carrington for the AGO1 and AGO2 tagged lines; H. Vaucheret for *ago1-27* mutant; M. J. Marcote for *mpk1 mpk2* seeds; and P. Karlovsky for pPK2 binary vector and

A. tumefaciens strain AGL1 for *B. cinerea* transformation and gene knockout. This work was supported by an NIH grant (R01 GM093008), an NSF Career Award (MCB-0642843), an NSF award (IOS-1257576), and an AES-CE award (PPA-7517H) to H.J.; National Science Council of the Republic of China (NSC 101-2311-B-009-003-MY3 and NSC 100-2627-B-009-002) and UST-UCSD I-RiCE Program (NSC 101-2911-I-009-101) grants to H.-D.H.

Supplementary Materials

www.sciencemag.org/content/342/6154/118/suppl/DC1
Materials and Methods
Fig. S1 to S10
Tables S1 to S4
References (29–32)

26 April 2013; accepted 19 August 2013
10.1126/science.1239705

Crystal Structure of Na⁺, K⁺-ATPase in the Na⁺-Bound State

Maria Nyblom,^{1,2,*†} Hanne Poulsen,^{1,2,3,*‡} Pontus Gourdon,^{1,2,3,*} Linda Reinhard,^{1,2,§} Magnus Andersson,⁴ Erik Lindahl,^{4,5} Natalya Fedosova,^{1,6} Poul Nissen^{1,2,3,†}

The Na⁺, K⁺-adenosine triphosphatase (ATPase) maintains the electrochemical gradients of Na⁺ and K⁺ across the plasma membrane—a prerequisite for electrical excitability and secondary transport. Hitherto, structural information has been limited to K⁺-bound or ouabain-blocked forms. We present the crystal structure of a Na⁺-bound Na⁺, K⁺-ATPase as determined at 4.3 Å resolution. Compared with the K⁺-bound form, large conformational changes are observed in the α subunit whereas the β and γ subunit structures are maintained. The locations of the three Na⁺ sites are indicated with the unique site III at the recently suggested IIIb, as further supported by electrophysiological studies on leak currents. Extracellular release of the third Na⁺ from IIIb through IIIa, followed by exchange of Na⁺ for K⁺ at sites I and II, is suggested.

The Na⁺, K⁺-adenosine triphosphatase (ATPase) is typically a ternary complex of a large catalytic α subunit associated with two smaller subunits, β and γ (Fig. 1A). Different isoforms combine to form kinetically distinct complexes in different cells and tissues (*1*). During the ATP-driven transport cycle, three cytoplasmic Na⁺ are exported in exchange for two extracellular K⁺ through alternating E1/E1P and E2P/E2 states (Fig. 1B), where E1 and E2 denote high affinity for

sodium and potassium ions, respectively, and P is phosphorylated. Intracellular Na⁺ and ATP binding stimulate phosphorylation of a conserved aspartic acid residue (Asp³⁶⁹ in pig α₁ isoform; see alignment in fig. S1), forming the sodium-occluded [Na₃]E1P-adenosine diphosphate (ADP) state. Conformational changes and ADP release open an extracellular pathway in the E2P state, and Na⁺ release and K⁺ binding stimulate dephosphorylation, yielding the potassium-occluded [K₂]E2P_i and [K₂]E2 states. Subsequent ATP binding and cytoplasmic K⁺ release lead to the sodium-bound E1 states.

Previously, K⁺-bound crystal structures representing the occluded E2P_i state as mimicked by magnesium fluoride ([K₂]E2-MgF_x) (2, 3) and structures with the inhibitory steroid ouabain (low-affinity [K₂]E2-MgF_x and high-affinity [Mg]E2P) have been elucidated (4, 5). The intracellular C-terminal tail of the α subunit plays an important role in Na⁺ binding (2, 6–10), apparently by controlling an ion pathway (6, 7), through a mechanism affected by many of the α₂ and α₃ isoform mutations that are associated with neurological diseases (11, 12). However, further elucidation of the transport mechanism of the Na⁺, K⁺-ATPase has been limited by the lack of a structure of the Na⁺-bound state, and in particular the location of the third Na⁺ site has remained unsettled.

We present here the crystal structure determined at 4.3 Å resolution of the Na⁺, K⁺-ATPase in the [Na₃]E1P-ADP state (pig renal α₁β₁γ enzyme) as mimicked by an ADP-AlF₄[−] complex (materials and methods and table S1) for which Na⁺ saturation was further stabilized by the presence of oligomycin. The structure was determined from an unbiased electron density map derived by single isomorphous replacement with anomalous scattering (SIRAS), using hexatantalum dodecaboride (Ta₆Br₁₂) derivatized crystals, followed by density modification procedures (Fig. 1C and fig. S2). Model building using sharpened maps and restrained refinement produced a final model with *R*_{work} and *R*_{free} of 26.1 and 28.8%, respectively. The structure represents two nearly identical complexes in the asymmetric unit (chains A-B-G and C-D-E) and displays bilayer features in the electron density (figs. S2 to S4).

The ability of the E1-AlF₄[−]-ADP complex to occlude three Na⁺ under crystallization-like conditions was confirmed by time-course measurements of ²²Na⁺ deocclusion at 0°C (Fig. 1F). The monoexponential fit resulted in the maximal number of 2.5 nmol of Na⁺ per nmol of ADP binding sites (i.e., 83% occupancy) at 1 mM ²²Na⁺ and the deocclusion rate constant of 0.02 s^{−1}. Assuming a Hill coefficient of 3 for the cooperative Na⁺ binding, the ion concentration required for the half-maximal saturation of the sites (*K*_{0.5} for Na⁺) was calculated to be 0.58 mM, consistent with previous findings (13). Thus, Na⁺ concentration under crystallization conditions (>80 mM) was more than two orders of magnitude higher than the *K*_{0.5}, enough to saturate all three sites.

The α subunit represents a Na⁺-occluded form of the transmembrane (TM) domain, with the cytoplasmic A, P, and N domains arranged for phosphorylation (Fig. 1, C and D) as observed for sarcoplasmic reticulum Ca²⁺ ATPase 1a (SERCA1a) in the equivalent, Ca²⁺-occluded state (fig. S4B) (14–16). Thus, compared with K⁺-bound forms, the α subunit is characterized by a different organization of the TM helices and a compact configuration of the cytoplasmic domains activating the phosphorylation site (Fig. 1, C and D, and fig. S5). Relative to the P domain, the A domain has undergone a rigid-body rota-

¹Centre for Membrane Pumps in Cells and Disease—PUMPKin, Danish National Research Foundation, DK-8000 Aarhus, Denmark.

²Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, DK-8000 Aarhus, Denmark. ³Danish Research Institute for Translational Neuroscience—DANDRITE, Nordic-EMBL Partnership of Molecular Medicine, Aarhus, Denmark.

⁴Science for Life Laboratory, Theoretical and Computational Biophysics, Department of Theoretical Physics, Swedish e-Science Research Center, KTH Royal Institute of Technology, SE-171 21 Solna, Sweden. ⁵Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden. ⁶Department of Biomedicine, Aarhus University, Ole Worms Allé 4, Building 1182, DK-8000 Aarhus, Denmark.

*These authors contributed equally to this work.

†Present address: Novo Nordisk A/S, Novo Nordisk Park, B9.2.31, DK-2760 Maalov, Denmark.

‡Corresponding author. E-mail: hp@mb.au.dk (H.P.); pn@mb.au.dk (P.N.)

§Present address: Department of Cell and Molecular Biology, Karolinska Institutet, Box 285, SE-171 77 Stockholm, Sweden.

tion and translation, displacing by 45 Å the TGES (T, Thr; G, Gly; E, Glu; S, Ser) motif involved in dephosphorylation of Asp³⁶⁹. Likewise, the N domain has rotated about 80° toward the P domain, stabilized by ADP-AlF₄⁻ at their interface (fig. S6). The P domain has rotated about 35° relative to the αM7 to αM10 (αM7-10) segment and moved together with the αM6-7 loop closer toward the TM region at the αC-terminal region (fig. S5).

The TM domain, which harbors the ion-binding sites and transport pathway, is formed by TM segments αM1-10, βM, and γM. Whereas αM7 to αM10 form a more rigid unit, αM1-αM6 are associated with significant structural rearrangements between K⁺- and Na⁺-bound conformations (Fig. 1 and fig. S7). The αM1-2 pair undergoes a rigid-body rotation toward αM9, the αM3-4 pair moves toward αM5-6 and translates toward the cytoplasmic side, whereas the cytoplasmic ends of αM5-6 move slightly toward the P domain with the extracellular portions nearly unchanged. This coupling of phosphorylation and occlusion of ion-binding sites at the TM domain, as com-

municated via tertiary interaction and connecting linkers, is the basis for the Na⁺ specificity of the intramembranous ion-binding sites that characterizes the E1P state.

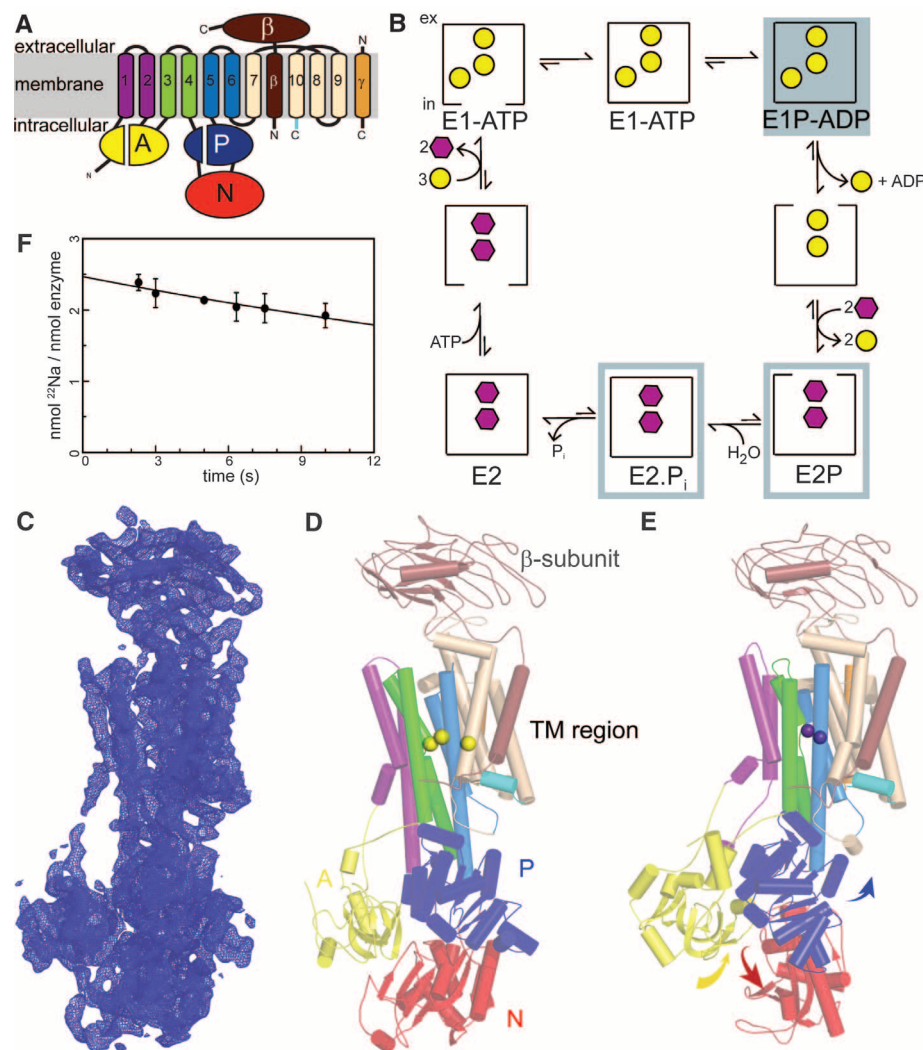
The β subunit has been proposed to undergo large conformational rearrangements relative to the α and γ subunits as part of the E2-to-E1 transition (17). However, in the [Na₃]E1-AlF₄⁻-ADP structure presented here, the β and γ subunits are not significantly changed compared to E2 states. The subunits are associated with the αM7-10 unit (figs. S1, S8, and S9), which is also seen to be fairly stable in SERCA1a (18, 19). However, conformational changes of the quaternary structure may occur in the E1 intermediate state, which was recently described for the sarcolipin-bound complex of SERCA1a (18, 19) (see below).

The Na⁺, K⁺-ATPase can bind three Na⁺ at intramembranous binding sites (20), of which sites I and II have been localized to αM4-6 (21) by mutational studies and homology modeling based on the two Ca²⁺ sites of SERCA1a (15, 16). In contrast, the binding position of the third Na⁺ has remained enigmatic; two locations have been pro-

posed on the basis of biochemical and electrophysiological studies: site IIIa between αM6, 8, and 9 and site IIIb between αM5, 7, and 8 (6, 7, 22–24) (Fig. 2A). Bound Na⁺ cannot be directly observed at 4.3 Å resolution, but ion-binding sites often show characteristic features even at low resolution because of the coordinated assembly of side chains and ions that concentrate x-ray scatterers between transmembrane helices. This is evident for the expected Na⁺ sites I and II, where we observe electron density compatible with occupancy at two sites between αM4 and αM6 (Fig. 2B and fig. S10). These sites are overall equivalent to the Ca²⁺ sites of SERCA1a (15), and the same residues also form the two K⁺ sites in the [K₂]E2-P_i state (4, 11) (Fig. 2, C and D).

The Na⁺ site III is specific to the Na⁺, K⁺-ATPase, and we are not guided by SERCA1a homology. However, a notable electron density feature, reminiscent of that observed for sites I and II, is observed at the proposed site IIIb with residues Thr⁷⁷⁴, Gln⁸⁵⁴, Gln⁹²³, and Asp⁹²⁶ between αM5, 7, and 8. In contrast, site IIIa, located adjacently with residues Glu⁹⁵⁴, Tyr⁷⁷¹ and Thr⁸⁰⁷,

Fig. 1. Crystal structure of the Na⁺, K⁺-ATPase with bound sodium ions. (A) The α subunit is the major catalytic unit and contains three cytoplasmic domains: the A (actuator, yellow), N (nucleotide binding, red), and P (phosphorylation, blue) (2). The TM region encompassing 10 TM helices (αM1-10) harbors the ion-binding sites. The C-terminal helices αM7-10 are shown in wheat and the αC terminus in cyan. The mobile TM fragments, αM1-2, αM3-4, and αM5-6, are colored purple, green, and blue, respectively. The β subunit (in red-brown) has one TM helix (βM) and a highly glycosylated extracellular domain, and it is associated with αM7 and αM10. (B) Overview of the functional cycle of the Na⁺, K⁺-ATPase. Yellow circles and purple hexagons represent Na⁺ and K⁺ ions, respectively. E1P/E2P indicate the phosphoenzyme intermediates. Closed boxes represent occluded states, and ex and in are short for extracellular and intracellular, respectively. The gray boxes mark previously determined structures (2–4, 32) (for E2P with ouabain), and the gray shaded box the state described here. (C) Final 2F_o – F_c electron density map of one αβγ complex (chains A, B, G) of the [Na₃]E1-AlF₄⁻-ADP crystal structure, displayed at 1.5σ contour level (blue mesh). (D) Cartoon representation in the same orientation as in (C) with the protein colored as in (A) and Na⁺ as yellow spheres. (E) Cartoon representation of the [K₂]E2-MgF_x structure aligned with αM7-10 of the [Na₃]E1P-ADP state, colored as in (A), and with K⁺ as purple spheres. The movements of the cytoplasmic domains relative to the [Na₃]E1P-ADP state are indicated by colored arrows. (F) Time dependence of ²²Na⁺ dissociation from the pig kidney enzyme preincubated with 1 mM ²²Na⁺ in the presence of oligomycin, ADP, and AlF₄⁻. The molar ratio of 2.5 obtained by extrapolation of the curve to time 0 argues for full saturation of all three sites at crystallization conditions (>80 mM Na⁺). Error bars indicate the standard deviation based on three independent experiments.



shows no indications of being coordinated as a Na^+ -bound site (figs. S10 and S11). Thus, we infer that site IIIb corresponds to Na^+ site III (Fig. 2, B and C). In support, two independent 45-ns molecular dynamics (MD) simulations yield a stable structure with Na^+ bound at site IIIb (fig. S12).

Mutational studies were previously unable to distinguish unambiguously between IIIa and IIIb, because mutation at either site lowers the rate of extracellular sodium rebinding to a similar extent (6); sodium affinity, though, is lowered more for IIIb mutants (6, 7, 22–24). To shed light on the roles of sites IIIa and IIIb, we performed electrophysiological studies of a characteristic leak current, which is tightly coupled to both sites (6, 24). The leak is an inwardly rectifying current, blocked by physiological levels of sodium or potassium and sensitive to the pump inhibitor ouabain (25). The leak is generally reduced by mutations in site IIIa (24), and it depends critically on Asp⁹²⁶ of IIIb; the Asp→Asn at position 926 (Asp926Asn) mutation blocks the leak at attainable voltages, presumably with the asparagine mimicking a constitutively protonated aspartate (6).

We examined the effects of mutations in either IIIa or IIIb more closely. Both of the IIIa mutants, Tyr771Phe and Glu954Ala, behaved similar to wild type: They leaked in the absence of extracellular sodium (albeit to a lesser extent), and the leak was closed by extracellular sodium

(Fig. 3). In contrast, mutating Asp⁹²⁶ of IIIb to alanine gave no measurable leak in the absence of sodium but tiny leaks in the presence of sodium, whereas both Asp926Glu and Glu854Asn (IIIb) leaked with and without sodium.

Thus, although mutation of either site causes slower sodium rebinding and lower affinity (6), there is a discernible difference in the roles that the two sites play in leaking. IIIb mutants are less sensitive to extracellular sodium, whereas IIIa mutations cause less leakage overall (Fig. 3). This is consistent with the two sites acting in concert with Na^+ binding tightly at IIIb and IIIa being a transient site for extracellular sodium release and rebinding as well as for the leak current. A mutated IIIa would therefore have lower affinity, especially for extracellular sodium, but once bound Na^+ at site IIIb would be favorably coordinated and also block the leak of IIIa mutants (Fig. 3). Consistent with a transient role for site IIIa, it was recently shown that deletion of a helical turn including Glu⁹⁵⁴ maintained forward pumping (26), albeit at a reduced level, which suggests that a 3:2 ratio of sodium to potassium is most likely possible even with a highly compromised site IIIa.

Binding of the third Na^+ at IIIb is expected to block the leak, because it depends on Asp⁹²⁶ as a stepping stone, raising the question whether sodium can escape the mutated site and leak into the cytoplasm (Fig. 4A). Binding of the third Na^+ is strongly voltage dependent, so hyperpolarizing

potentials favor occupancy at site IIIb, and, if Na^+ were not able to leak from a mutated site IIIb, increasingly hyperpolarizing potentials would be expected to eliminate leaking in the presence of sodium. However, the opposite is observed, which favors that a destabilized IIIb allows Na^+ to slip past the Asp⁹²⁶ checkpoint and enter the cytoplasm.

The exchange of Na^+ between the stable IIIb and a transient site IIIa would be dynamic and associated with conformational changes that allow extracellular release from IIIa. Additionally, Glu⁹⁵⁴ of IIIa is located at the α M2, 6, and 9 cleft, which binds sarcolipin in SERCA (18, 19), and the neighboring Glu⁹⁵³ interacts directly with the γ subunit associated with α M9 (Fig. 4B). We find it likely that γ subunit and other proteins of the so-called FXYD family (where F is Phe; X, any amino acid, Y, Tyr; D, Asp) exert their regulatory function on Na^+ , K^+ -ATPase activity by interference with site IIIa function.

Several mutations affecting the C-terminal structure encompassing site IIIb of the α_2 and α_3 isoforms have been observed for the neurological diseases rapid-onset dystonia with parkinsonism (RDP) (12), hemiplegic migraines (27), and alternating hemiplegia of childhood (AHC) (28, 29). Particularly in AHC, the ion-binding sites are hot spots. More than a third of the cases are linked to mutation of α_3 Asp⁸⁰¹ (equivalent of pig α_1 Asp⁸⁰⁴) between sites I and II to an asparagine (28, 29), but there is also a case of the IIIb aspartate (the

Fig. 2. The three Na^+ sites in the transmembrane domain. The protein is seen from the cytoplasmic side, about 90° rotated and colored as in Fig. 1. Key residues shown as sticks. (A) Schematic representation of the TM helices in the $[\text{Na}_3]\text{E1P-ADP}$ state. The position and numbering of the proposed Na^+ sites (yellow spheres) are indicated. (B) For Na^+ -binding sites I, II, and IIIb, unbiased electron density is visible between TM helices as seen from $F_o - F_c$ (contoured at $\pm 4.5\sigma$, green/red mesh) and $2F_o - F_c$ maps (contoured at 2.0σ , blue mesh; see figs. S10 to S12 for further information). (C) Ion-binding residues in the $[\text{Na}_3]\text{E1-ATP}_4^-$ -ADP form. Sodium ion positions are not accurate at the resolution of this study. TM helices are shown in white for clarity. Q, Gln. (D) Equivalent view as (C) for the $[\text{K}_2]\text{E2-MgF}_x$ form (PDB code 3KDP).

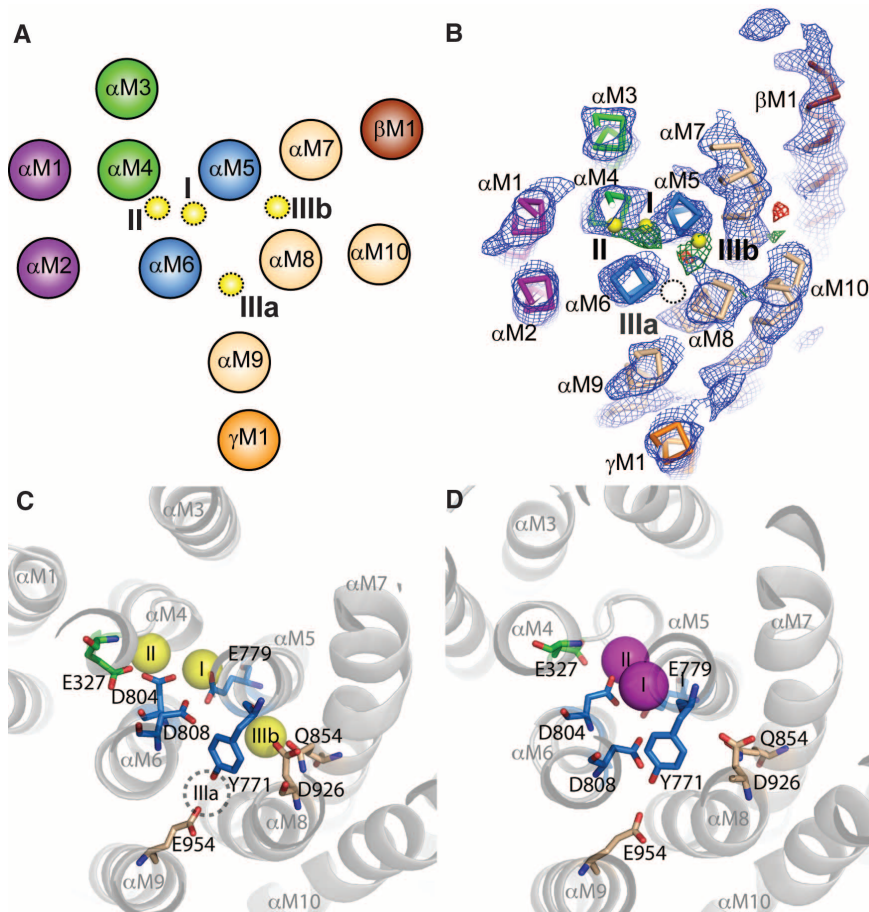


Fig. 3. Steady-state currents and leak currents of IIIa and IIIb mutants. Pump current is calculated as the ouabain-sensitive current in the presence of 15 mM potassium; the leak current is determined as the ouabain-sensitive current in the absence of potassium. Both pump and leak currents are normalized to the value of pump current at 20 mV. Pump currents in the presence (round black) and absence (gray up triangle) of 115 mM sodium as well as leak currents in the presence (square black) and absence (gray down triangle) of 115 mM sodium are shown. Error bars, SEM; $n = 3$ to 6 oocytes. Residue numbering corresponds to the pig α_1 isoform. Average of currents at 20 mV with/without sodium used for normalizations: E954A (A, Ala), 139 nA/101 nA; Y771F, 81 nA/109 nA; D926A, 92 nA/105 nA; D926E, 69 nA/61 nA; Q854N (N, Asn), 60 nA/61 nA.

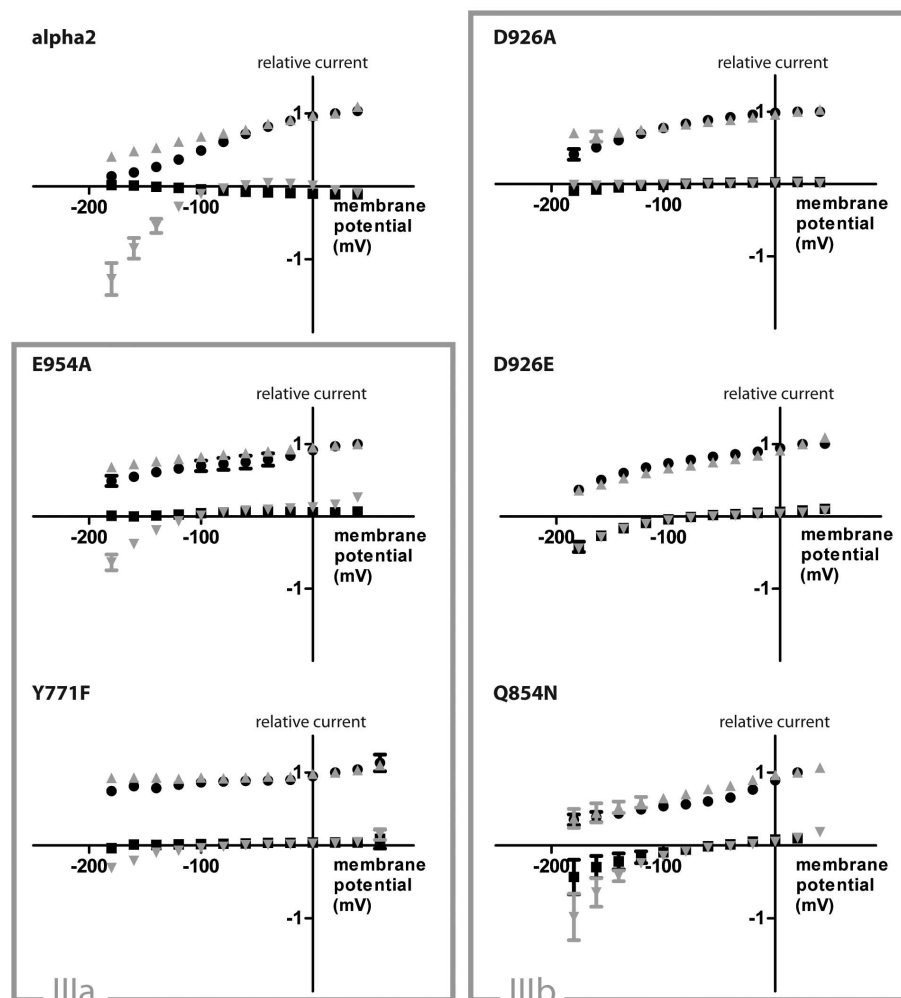
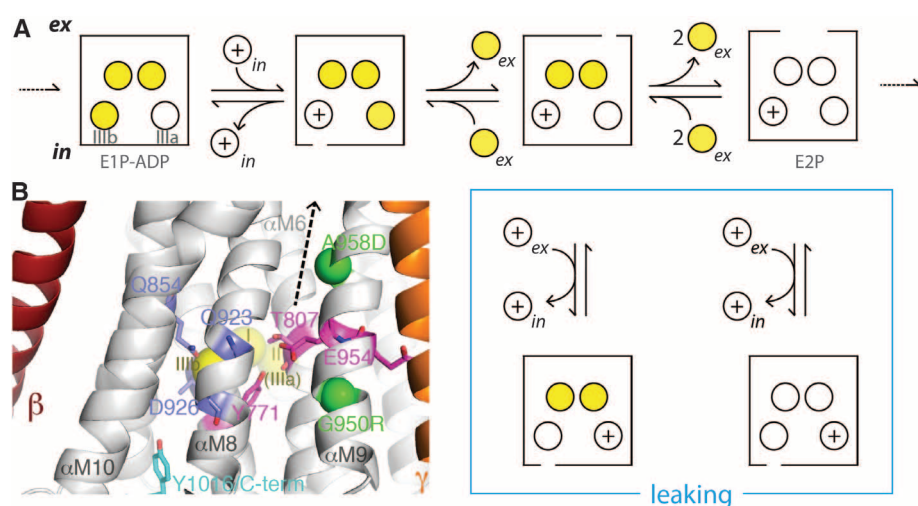


Fig. 4. Model for sodium release from Na^+ , K^+ -ATPase. (A) Schematic model illustrating extracellular sodium release and leaking. Na^+ , yellow circle; proton, $\oplus$. The forward reaction is toward the right (full cycle in Fig. 1B). Extracellular release of Na^+ from IIIb is associated with protonation of Asp⁹²⁶ in a voltage-dependent reaction, so hyperpolarizing potentials promote the reverse reaction of Na^+ binding from the extracellular side to IIIb. The steps causing release of the other two sodium ions are less voltage sensitive (20). In the blue box, the proposed mechanism for proton leaking is indicated: An extracellular proton can bind site IIIa either in the presence of Na^+ at sites I and II (left) or in the absence of sodium (right), the IIIb proton is released intracellularly, and transfer of the IIIa proton to IIIb makes the site accessible for an extracellular proton again. Repeated extracellular binding and intracellular release of protons will create a leaking current. (B) Cartoon representation of the Na^+ -binding sites (Na^+ in yellow spheres). Site IIIa and IIIb residues and the C terminus are indicated in magenta, blue, and cyan sticks, respectively, and the position of two AHC mutations by green spheres. A possible initial Na^+ release pathway through a transient site IIIa is indicated by a dashed arrow. Note the link between the γ subunit interface and site IIIa.



Asp⁹²⁶ here) mutated to a tyrosine (29). Mutation of the IIIb aspartate to asparagine was previously linked to RDP (30, 31). Furthermore, two cases of AHC likely have perturbed IIIa function because the mutations are both one helical turn

from Glu⁹⁵⁴ and cause a neutral residue to be substituted for a charged residue (Gly950Arg and Ala958Asp, pig α_1 residue numbers, Fig. 4B).

Proper binding and release of the third and electrogenic Na^+ of the Na^+ , K^+ -ATPase is es-

sential for pump function, and the crystallographic and electrophysiological data presented here support a model in which Asp⁹²⁶ is the central ion-coordinating residue at site III as controlled by the C-terminal region and that Glu⁹⁵⁴ of a cooperative

site IIIa is associated with Na⁺ release. High-resolution structures that accurately reveal Na⁺ coordination and associated hydrogen-bonding networks will be essential for a better understanding of the structure-function relations of ion exchange, transport, and specificity and how the mechanism is affected by regulation and disease-related mutations.

References and Notes

1. G. Blanco, R. W. Mercer, *Am. J. Physiol.* **275**, F633–F650 (1998).
2. J. P. Morth *et al.*, *Nature* **450**, 1043–1049 (2007).
3. T. Shinoda, H. Ogawa, F. Cornelius, C. Toyoshima, *Nature* **459**, 446–450 (2009).
4. H. Ogawa, T. Shinoda, F. Cornelius, C. Toyoshima, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13742–13747 (2009).
5. M. Laursen, L. Yatime, P. Nissen, N. U. Fedosova, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 10958–10963 (2013).
6. H. Poulsen *et al.*, *Nature* **467**, 99–102 (2010).
7. A. P. Einholm, M. S. Toustrup-Jensen, R. Holm, J. P. Andersen, B. Vilens, *J. Biol. Chem.* **285**, 26245–26254 (2010).
8. S. Meier, N. N. Tavaraz, K. L. Dürr, T. Friedrich, *J. Gen. Physiol.* **135**, 115–134 (2010).
9. N. Vedovato, D. C. Gadsby, *J. Gen. Physiol.* **136**, 63–82 (2010).
10. M. S. Toustrup-Jensen *et al.*, *J. Biol. Chem.* **284**, 18715–18725 (2009).
11. P. Blanco-Arias *et al.*, *Hum. Mol. Genet.* **18**, 2370–2377 (2009).
12. P. de Carvalho Aguiar *et al.*, *Neuron* **43**, 169–175 (2004).
13. M. Esmann, J. C. Skou, *Biochem. Biophys. Res. Commun.* **127**, 857–863 (1985).
14. C. Olesen *et al.*, *Nature* **450**, 1036–1042 (2007).
15. T. L. Sørensen, J. V. Møller, P. Nissen, *Science* **304**, 1672–1675 (2004).
16. C. Toyoshima, T. Mizutani, *Nature* **430**, 529–535 (2004).
17. R. E. Dempsey, K. Hartung, T. Friedrich, E. Bamberg, *J. Biol. Chem.* **281**, 36338–36346 (2006).
18. A. M. Winther *et al.*, *Nature* **495**, 265–269 (2013).
19. C. Toyoshima *et al.*, *Nature* **495**, 260–264 (2013).
20. M. Holmgren *et al.*, *Nature* **403**, 898–901 (2000).
21. H. Ogawa, C. Toyoshima, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15977–15982 (2002).
22. T. Imagawa, T. Yamamoto, S. Kaya, K. Sakaguchi, K. Taniguchi, *J. Biol. Chem.* **280**, 18736–18744 (2005).
23. E. A. Jewell-Motz, J. B. Lingrel, *Biochemistry* **32**, 13523–13530 (1993).
24. C. Li, K. Geering, J. D. Horisberger, *J. Membr. Biol.* **213**, 1–9 (2006).
25. A. Vasilyev, K. Khater, R. F. Rakowski, *J. Membr. Biol.* **198**, 65–76 (2004).
26. E. A. Azizan *et al.*, *Nat. Genet.* **45**, 1055–1060 (2013).
27. M. De Fusco *et al.*, *Nat. Genet.* **33**, 192–196 (2003).
28. E. L. Heinzen *et al.*, *Nat. Genet.* **44**, 1030–1034 (2012).
29. H. Rosewich *et al.*, *Lancet Neurol.* **11**, 764–773 (2012).
30. I. A. Anselm, K. J. Sweadner, S. Gollamudi, L. J. Ozelius, B. T. Darras, *Neurology* **73**, 400–401 (2009).
31. P. Zanotti-Fregonara *et al.*, *J. Neurol. Sci.* **273**, 148–151 (2008).
32. L. Yatime *et al.*, *J. Struct. Biol.* **174**, 296–306 (2011).

Acknowledgments: B. Vilens and J. Petersen, Department of Biomedicine, Aarhus University, Denmark, are thanked

for preparing enzyme for crystallization. We thank C. Schulze-Bries, T. Tomizaki, and V. Olieric (Swiss Light Source) for assistance with synchrotron data collection; B. Bjerring Jensen, A. M. Nielsen, and J. L. Karlén for technical assistance; and J. P. Morth, L. Yatime, M. Laursen, H. Khandelia, and M. J. Clausen for valuable discussions. Support was provided by the Dansk program of the Danish Natural Science Research Council. M.N. was supported by the Swedish Research Council, L.R. by the Danish Council for Independent Research in Medical Sciences, E.L. by a European Research Council starting grant (contract 209825), and P.N. by a European Research Council advanced grant (contract 250322). H.P. was supported by the Lundbeck Foundation, the Carlsberg Foundation, and L'Oréal/United Nations Educational, Scientific, and Cultural Organization. The authors made the following contributions: H.P. and P.N. performed study design. M.N. crystallized the protein, collected and processed x-ray data, and determined and refined the structure, assisted by L.R. and P.G. The structural analysis was carried out by M.N., L.R., and P.G., assisted by P.N., whereas M.A. and E.L. performed the MD simulations, assisted by P.G. H.P. designed and performed the electrophysiological studies. N.F. designed and performed the deocclusion experiments. M.N., L.R., P.G., H.P., and P.N. wrote the paper. All authors discussed the results and commented on the manuscript. Coordinates and structure factors have been deposited in the Protein Data Bank (PDB) with accession no. 4hqj.

Supplementary Materials

www.sciencemag.org/content/342/6154/123/suppl/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References (33–58)

17 July 2013; accepted 3 September 2013
10.1126/science.1243352

Quantifying Long-Term Scientific Impact

Dashun Wang,^{1,2*} Chaoming Song,^{1,3*} Albert-László Barabási^{1,4,5,6,†}

The lack of predictability of citation-based measures frequently used to gauge impact, from impact factors to short-term citations, raises a fundamental question: Is there long-term predictability in citation patterns? Here, we derive a mechanistic model for the citation dynamics of individual papers, allowing us to collapse the citation histories of papers from different journals and disciplines into a single curve, indicating that all papers tend to follow the same universal temporal pattern. The observed patterns not only help us uncover basic mechanisms that govern scientific impact but also offer reliable measures of influence that may have potential policy implications.

Of the many tangible measures of scientific impact, one stands out in its frequency of use: citations (1–10). The reliance on citation-based measures, from the Hirsch index (4) to the g-index (11), from impact factors (1) to eigenfactors (12), and on diverse ranking-based

metrics (13) lies in the (often debated) perception that citations offer a quantitative proxy of a discovery's importance or a scientist's standing in the research community. Often lost in this debate is the fact that our ability to foresee lasting impact on the basis of citation patterns has well-known limitations.

1) The impact factor (IF) (1), conferring a journal's historical impact to a paper, is a poor predictor of a particular paper's future citations (14, 15): Papers published in the same journal a decade later acquire widely different number of citations, from one to thousands (fig. S2A).

2) The number of citations (2) collected by a paper strongly depends on the paper's age; hence, citation-based comparisons favor older papers and established investigators. It also lacks predictive

power: A group of papers that within a 5-year span collect the same number of citations are found to have widely different long-term impacts (fig. S2B).

3) Paradigm-changing discoveries have notoriously limited early impact (3), precisely because the more a discovery deviates from the current paradigm, the longer it takes to be appreciated by the community (16). Indeed, although for most papers their early- and long-term citations correlate, this correlation breaks down for discoveries with the most long-term citations (Fig. 1B). Hence, publications with exceptional long-term impact appear to be the hardest to recognize on the basis of their early citation patterns.

4) Comparison of different papers is confounded by incompatible publication, citation, and/or acknowledgment traditions of different disciplines and journals.

Long-term cumulative measures like the Hirsch index have predictable components that can be extracted via data mining (4, 17). Yet, given the myriad of factors involved in the recognition of a new discovery, from the work's intrinsic value to timing, chance, and the publishing venue, finding regularities in the citation history of individual papers, the minimal carriers of a scientific discovery, remains an elusive task.

In the past, much attention has focused on citation distributions, with debates on whether they follow a power law (2, 18, 19) or a log-normal form (3, 7, 15). Also, universality across disciplines allowed the rescaling of the distributions

¹Center for Complex Network Research, Department of Physics, Department of Biology, and Department of Computer Science, Northeastern University, Boston, MA 02115, USA. ²IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598, USA. ³Department of Physics, University of Miami, Coral Gables, FL 33124, USA. ⁴Center for Cancer Systems Biology, Dana Farber Cancer Institute, Boston, MA 02115, USA. ⁵Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. ⁶Center for Network Science, Central European University, Budapest, Hungary.

*These authors contributed equally to the work.

†Corresponding author. E-mail: alb@neu.edu



Quantifying Long-Term Scientific Impact

Dashun Wang *et al.*

Science **342**, 127 (2013);

DOI: 10.1126/science.1237825

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of October 3, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/342/6154/127.full.html>

Supporting Online Material can be found at:

<http://www.sciencemag.org/content/suppl/2013/10/02/342.6154.127.DC1.html>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/content/342/6154/127.full.html#related>

This article **cites 39 articles**, 16 of which can be accessed free:

<http://www.sciencemag.org/content/342/6154/127.full.html#ref-list-1>

This article has been **cited by 1** articles hosted by HighWire Press; see:

<http://www.sciencemag.org/content/342/6154/127.full.html#related-urls>

site IIIa is associated with Na⁺ release. High-resolution structures that accurately reveal Na⁺ coordination and associated hydrogen-bonding networks will be essential for a better understanding of the structure-function relations of ion exchange, transport, and specificity and how the mechanism is affected by regulation and disease-related mutations.

References and Notes

1. G. Blanco, R. W. Mercer, *Am. J. Physiol.* **275**, F633–F650 (1998).
2. J. P. Morth *et al.*, *Nature* **450**, 1043–1049 (2007).
3. T. Shinoda, H. Ogawa, F. Cornelius, C. Toyoshima, *Nature* **459**, 446–450 (2009).
4. H. Ogawa, T. Shinoda, F. Cornelius, C. Toyoshima, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13742–13747 (2009).
5. M. Laursen, L. Yatime, P. Nissen, N. U. Fedosova, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 10958–10963 (2013).
6. H. Poulsen *et al.*, *Nature* **467**, 99–102 (2010).
7. A. P. Einholm, M. S. Toustrup-Jensen, R. Holm, J. P. Andersen, B. Vilsen, *J. Biol. Chem.* **285**, 26245–26254 (2010).
8. S. Meier, N. N. Tavaraz, K. L. Dürr, T. Friedrich, *J. Gen. Physiol.* **135**, 115–134 (2010).
9. N. Vedovato, D. C. Gadsby, *J. Gen. Physiol.* **136**, 63–82 (2010).
10. M. S. Toustrup-Jensen *et al.*, *J. Biol. Chem.* **284**, 18715–18725 (2009).
11. P. Blanco-Arias *et al.*, *Hum. Mol. Genet.* **18**, 2370–2377 (2009).
12. P. de Carvalho Aguiar *et al.*, *Neuron* **43**, 169–175 (2004).
13. M. Esmann, J. C. Skou, *Biochem. Biophys. Res. Commun.* **127**, 857–863 (1985).
14. C. Olesen *et al.*, *Nature* **450**, 1036–1042 (2007).
15. T. L. Sørensen, J. V. Møller, P. Nissen, *Science* **304**, 1672–1675 (2004).
16. C. Toyoshima, T. Mizutani, *Nature* **430**, 529–535 (2004).
17. R. E. Dempsey, K. Hartung, T. Friedrich, E. Bamberg, *J. Biol. Chem.* **281**, 36338–36346 (2006).
18. A. M. Winther *et al.*, *Nature* **495**, 265–269 (2013).
19. C. Toyoshima *et al.*, *Nature* **495**, 260–264 (2013).
20. M. Holmgren *et al.*, *Nature* **403**, 898–901 (2000).
21. H. Ogawa, C. Toyoshima, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15977–15982 (2002).
22. T. Imagawa, T. Yamamoto, S. Kaya, K. Sakaguchi, K. Taniguchi, *J. Biol. Chem.* **280**, 18736–18744 (2005).
23. E. A. Jewell-Motz, J. B. Lingrel, *Biochemistry* **32**, 13523–13530 (1993).
24. C. Li, K. Geering, J. D. Horisberger, *J. Membr. Biol.* **213**, 1–9 (2006).
25. A. Vasilyev, K. Khater, R. F. Rakowski, *J. Membr. Biol.* **198**, 65–76 (2004).
26. E. A. Azizan *et al.*, *Nat. Genet.* **45**, 1055–1060 (2013).
27. M. De Fusco *et al.*, *Nat. Genet.* **33**, 192–196 (2003).
28. E. L. Heinzen *et al.*, *Nat. Genet.* **44**, 1030–1034 (2012).
29. H. Rosewich *et al.*, *Lancet Neurol.* **11**, 764–773 (2012).
30. I. A. Anselm, K. J. Sweadner, S. Gollamudi, L. J. Ozelius, B. T. Darras, *Neurology* **73**, 400–401 (2009).
31. P. Zanotti-Fregonara *et al.*, *J. Neurol. Sci.* **273**, 148–151 (2008).
32. L. Yatime *et al.*, *J. Struct. Biol.* **174**, 296–306 (2011).

Acknowledgments: B. Vilsen and J. Petersen, Department of Biomedicine, Aarhus University, Denmark, are thanked

for preparing enzyme for crystallization. We thank C. Schulze-Bries, T. Tomizaki, and V. Olieric (Swiss Light Source) for assistance with synchrotron data collection; B. Bjerring Jensen, A. M. Nielsen, and J. L. Karlén for technical assistance; and J. P. Morth, L. Yatime, M. Laursen, H. Khandelia, and M. J. Clausen for valuable discussions. Support was provided by the Danskatt program of the Danish Natural Science Research Council. M.N. was supported by the Swedish Research Council, L.R. by the Danish Council for Independent Research in Medical Sciences, E.L. by a European Research Council starting grant (contract 209825), and P.N. by a European Research Council advanced grant (contract 250322). H.P. was supported by the Lundbeck Foundation, the Carlsberg Foundation, and L'Oréal/United Nations Educational, Scientific, and Cultural Organization. The authors made the following contributions: H.P. and P.N. performed study design. M.N. crystallized the protein, collected and processed x-ray data, and determined and refined the structure, assisted by L.R. and P.G. The structural analysis was carried out by M.N., L.R., and P.G., assisted by P.N., whereas M.A. and E.L. performed the MD simulations, assisted by P.G. H.P. designed and performed the electrophysiological studies. N.F. designed and performed the deocclusion experiments. M.N., L.R., P.G., H.P., and P.N. wrote the paper. All authors discussed the results and commented on the manuscript. Coordinates and structure factors have been deposited in the Protein Data Bank (PDB) with accession no. 4hqj.

Supplementary Materials

www.sciencemag.org/content/342/6154/123/suppl/DC1
Materials and Methods
Figs. S1 to S12
Table S1
References (33–58)

17 July 2013; accepted 3 September 2013
10.1126/science.1243352

Quantifying Long-Term Scientific Impact

Dashun Wang,^{1,2*} Chaoming Song,^{1,3*} Albert-László Barabási^{1,4,5,6,†}

The lack of predictability of citation-based measures frequently used to gauge impact, from impact factors to short-term citations, raises a fundamental question: Is there long-term predictability in citation patterns? Here, we derive a mechanistic model for the citation dynamics of individual papers, allowing us to collapse the citation histories of papers from different journals and disciplines into a single curve, indicating that all papers tend to follow the same universal temporal pattern. The observed patterns not only help us uncover basic mechanisms that govern scientific impact but also offer reliable measures of influence that may have potential policy implications.

Of the many tangible measures of scientific impact, one stands out in its frequency of use: citations (1–10). The reliance on citation-based measures, from the Hirsch index (4) to the g-index (11), from impact factors (1) to eigenfactors (12), and on diverse ranking-based

metrics (13) lies in the (often debated) perception that citations offer a quantitative proxy of a discovery's importance or a scientist's standing in the research community. Often lost in this debate is the fact that our ability to foresee lasting impact on the basis of citation patterns has well-known limitations.

1) The impact factor (IF) (1), conferring a journal's historical impact to a paper, is a poor predictor of a particular paper's future citations (14, 15): Papers published in the same journal a decade later acquire widely different number of citations, from one to thousands (fig. S2A).

2) The number of citations (2) collected by a paper strongly depends on the paper's age; hence, citation-based comparisons favor older papers and established investigators. It also lacks predictive

power: A group of papers that within a 5-year span collect the same number of citations are found to have widely different long-term impacts (fig. S2B).

3) Paradigm-changing discoveries have notoriously limited early impact (3), precisely because the more a discovery deviates from the current paradigm, the longer it takes to be appreciated by the community (16). Indeed, although for most papers their early- and long-term citations correlate, this correlation breaks down for discoveries with the most long-term citations (Fig. 1B). Hence, publications with exceptional long-term impact appear to be the hardest to recognize on the basis of their early citation patterns.

4) Comparison of different papers is confounded by incompatible publication, citation, and/or acknowledgment traditions of different disciplines and journals.

Long-term cumulative measures like the Hirsch index have predictable components that can be extracted via data mining (4, 17). Yet, given the myriad of factors involved in the recognition of a new discovery, from the work's intrinsic value to timing, chance, and the publishing venue, finding regularities in the citation history of individual papers, the minimal carriers of a scientific discovery, remains an elusive task.

In the past, much attention has focused on citation distributions, with debates on whether they follow a power law (2, 18, 19) or a log-normal form (3, 7, 15). Also, universality across disciplines allowed the rescaling of the distributions

¹Center for Complex Network Research, Department of Physics, Department of Biology, and Department of Computer Science, Northeastern University, Boston, MA 02115, USA. ²IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598, USA. ³Department of Physics, University of Miami, Coral Gables, FL 33124, USA. ⁴Center for Cancer Systems Biology, Dana Farber Cancer Institute, Boston, MA 02115, USA. ⁵Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. ⁶Center for Network Science, Central European University, Budapest, Hungary.

*These authors contributed equally to the work.

†Corresponding author. E-mail: alb@neu.edu

by discipline-dependent variables (7, 15). Together, these results offer convincing evidence that the aggregated citation patterns are characterized by generic scaling laws. Yet little is known about the mechanisms governing the temporal evolution of individual papers. The inherent difficulty in addressing this problem is well illustrated by the citation history of papers extracted from the *Physical Review* (PR) corpus (Fig. 1A), consisting of 463,348 papers published between 1893 and 2010 and spanning all areas of physics (3). The fat-tailed nature of the citation distribution 30 years after publication indicates that, although most papers are hardly cited, a few do have exceptional impact (Fig. 1B, inset) (2, 3, 7, 19, 20). This impact heterogeneity, coupled with widely different citation histories (Fig. 1A), suggests a lack of order and hence lack of predictability in citation patterns. As we show next, this lack of order in citation histories is only apparent, because

citations follow widely reproducible dynamical patterns that span research fields.

We start by identifying three fundamental mechanisms that drive the citation history of individual papers:

Preferential attachment captures the well-documented fact that highly cited papers are more visible and are more likely to be cited again than less-cited contributions (20, 21). Accordingly a paper i 's probability to be cited again is proportional to the total number of citations c_i the paper received previously (fig. S3).

Aging captures the fact that new ideas are integrated in subsequent work; hence, each paper's novelty fades eventually (22, 23). The resulting long-term decay is best described by a log-normal survival probability (Fig. 1C and supplementary materials S2.1), where t is time; μ indicates immediacy, governing the time for a paper to reach its citation peak; and σ is longevity, capturing the decay rate.

$$P_i(t) = \frac{1}{\sqrt{2\pi\sigma_i^2 t}} \exp \left[-\frac{(\ln t - \mu_i)^2}{2\sigma_i^2} \right] \quad (1)$$

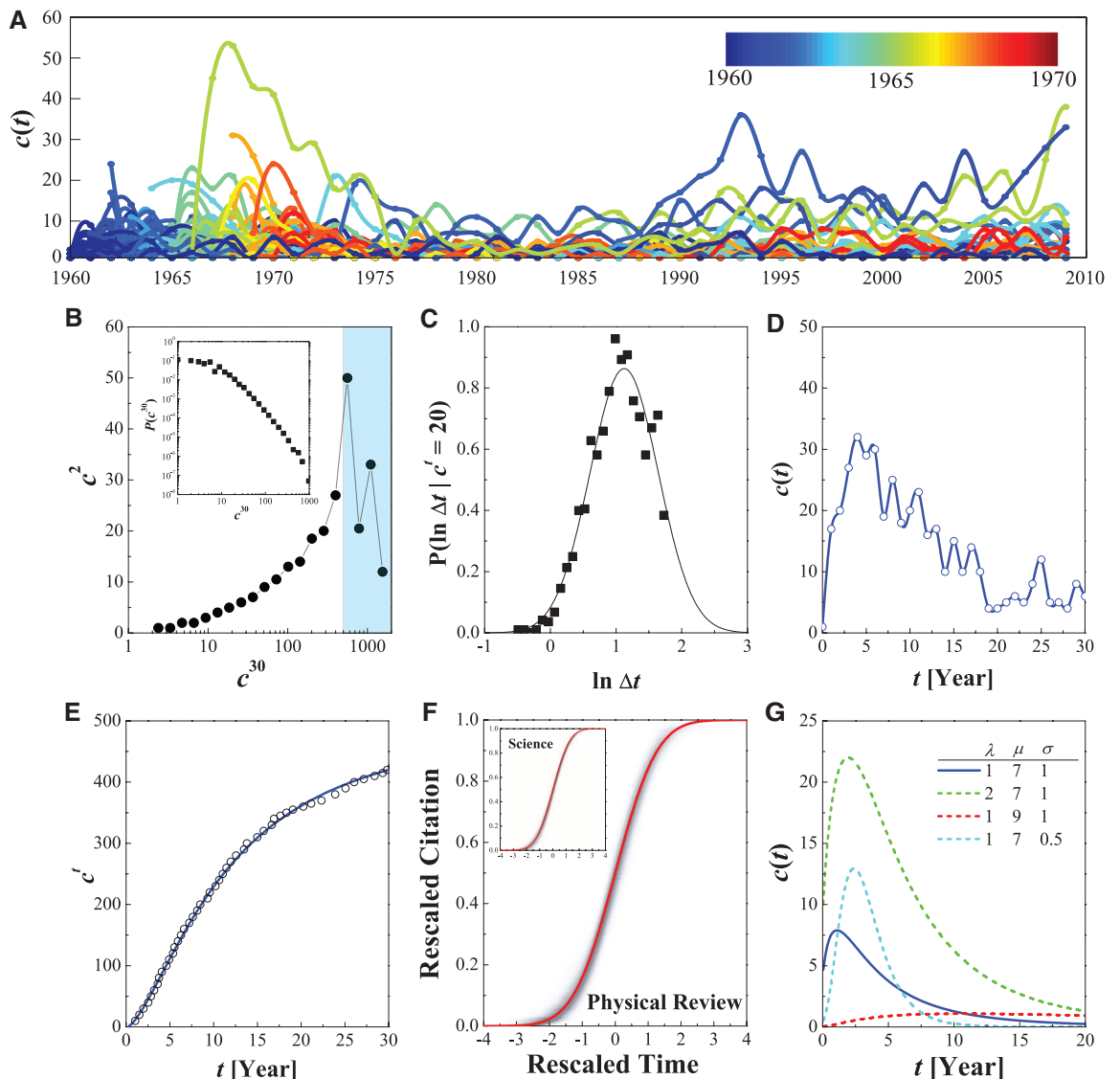
Fitness, η_i , captures the inherent differences between papers, accounting for the perceived novelty and importance of a discovery (24, 25). Novelty and importance depend on so many intangible and subjective dimensions that it is impossible to objectively quantify them all. Here, we bypass the need to evaluate a paper's intrinsic value and view fitness η_i as a collective measure capturing the community's response to a work.

Combining these three factors, we can write the probability that paper i is cited at time t after publication as

$$\Pi_i(t) \sim \eta_i c_i^{\lambda} P_i(t) \quad (2)$$

Solving the associated master equation, Eq. 2 allows us to predict the cumulative number of

Fig. 1. Characterizing citation dynamics. (A) Yearly citation $c_i(t)$ for 200 randomly selected papers published between 1960 and 1970 in the PR corpus. The color code corresponds to each papers' publication year. (B) Average number of citations acquired 2 years after publication (c^2) for papers with the same long-term impact (c^{30}), indicating that for high-impact papers ($c^{30} \geq 400$, shaded area) the early citations underestimate future impact. (Inset) Distribution of citations 30 years after publication (c^{30}) for PR papers published between 1950 and 1980. (C) Distribution of papers' ages when they get cited. To separate the effect of preferential attachment, we measured the aging function for papers with the same number of previous citations (here $c^t = 20$; see also supplementary materials S2.1). The solid line corresponds to a Gaussian fit of the data, indicating that $P(\ln \Delta t | c^t)$ follows a normal distribution. (D) Yearly citation $c(t)$ for a research paper from the PR corpus. (E) Cumulative citations c^t for the paper in (D) together with the best fit to Eq. 3 (solid line). (F) Data collapse for 7775 papers with more than 30 citations within 30 years in the PR corpus published between 1950 and 1980. (Inset) Data collapse for the 20-year citation histories of all papers published by *Science* in 1990 (842 papers). (G) Changes in the citation history $c(t)$ according to Eq. 3 after varying the λ , μ , and σ parameters, indicating that Eq. 3 can account for a wide range of citation patterns.



citations acquired by paper i at time t after publication (supplementary materials S2.2)

$$c_i^t = m \left[e^{\frac{\beta \mu_i}{A} \Phi\left(\frac{\ln t - \mu_i}{\sigma_i}\right)} - 1 \right] \equiv m \left[e^{\lambda_i \Phi\left(\frac{\ln t - \mu_i}{\sigma_i}\right)} - 1 \right] \quad (3)$$

where

$$\Phi(x) \equiv (2\pi)^{-1/2} \int_{-\infty}^x e^{-y^2/2} dy \quad (4)$$

is the cumulative normal distribution, m measures the average number of references each new paper

contains, β captures the growth rate of the total number of publications (supplementary materials S1.3), and A is a normalization constant (supplementary materials S2.2). Hence m , β , and A are global parameters, having the same value for all publications. We have chosen $m = 30$ throughout the paper, because our results do not depend on this choice (supplementary materials S2.3). Equation 3 represents a minimal citation model that captures all known quantifiable mechanisms that affect citation histories. It predicts that the citation history of paper i is characterized by three fundamental parameters: the relative fit-

ness, $\lambda_i \equiv \eta_i \beta / A$, capturing a paper's importance relative to other papers; μ_i ; and σ_i . By using the rescaled variables $\tilde{t} \equiv (\ln t - \mu_i) / \sigma_i$ and $\tilde{c} \equiv \ln(1 + c_i^t / m) / \lambda_i$, we obtain our main result

$$\tilde{c} = \Phi(\tilde{t}) \quad (5)$$

predicting that each paper's citation history should follow the same universal curve $\Phi(\tilde{t})$ if rescaled with the paper-specific (λ_i , μ_i , and σ_i) parameters. Therefore, given a paper's citation history, that is, t and c_i^t , we can obtain the best-fitted three parameters for paper i by using Eq. 3. To illustrate the process, we selected a paper from our corpus, whose citation history is shown in Fig. 1, D and E. We fitted to Eq. 3 the paper's cumulative citations (Fig. 1E) by using the least square fit method, obtaining $\lambda = 2.87$, $\mu = 7.38$, and $\sigma = 1.2$. To illustrate the validity of the fit, we show (Fig. 1E) the prediction of Eq. 3 using the uncovered fit parameters.

To test the model's validity, we rescaled all papers published between 1950 and 1980 in the *PR* corpus, finding that they all collapse into Eq. 5 (Fig. 1F, see also supplementary materials S2.4.1 for the statistical test of the data collapse). The reason is explained in Fig. 1G: By varying λ , μ , and σ , Eq. 3 can account for a wide range of empirically observed citation histories, from jump-decay patterns to delayed impact. We also tested our model on all papers published in 1990 by 12 prominent journals (table S4), finding an exceptional collapse for all (see Fig. 1G, inset, for *Science* and supplementary materials S2.4.2 and fig. S8 for the other journals).

The model Eqs. 3 to 5 also predicts several fundamental measures of impact:

Ultimate impact (c^∞) represents the total number of citations a paper acquires during its lifetime. By taking the $t \rightarrow \infty$ limit in Eq. 3, we obtain

$$c_i^\infty = m(e^{\lambda_i} - 1) \quad (6)$$

a simple formula that predicts that the total number of citations acquired by a paper during its lifetime is independent of immediacy (μ) or the rate of decay (σ) and depends only on a single parameter, the paper's relative fitness, λ .

Impact time (T_i^*) represents the characteristic time it takes for a paper to collect the bulk of its citations. A natural measure is the time necessary for a paper to reach the geometric mean of its final citations, obtaining (supplementary materials S2.2)

$$T_i^* \approx \exp(\mu_i) \quad (7)$$

Hence, impact time is mainly determined by the immediacy parameter μ_i and is independent of fitness λ_i or decay σ_i .

The proposed model offers a journal-free methodology to evaluate long term impact. To illustrate this, we selected three journals with widely different IFs: *Physical Review B* (*PRB*) (IF = 3.26 in 1992), *Proceedings of the National Academy of Sciences USA* (*PNAS*) (10.48), and *Cell* (33.62).

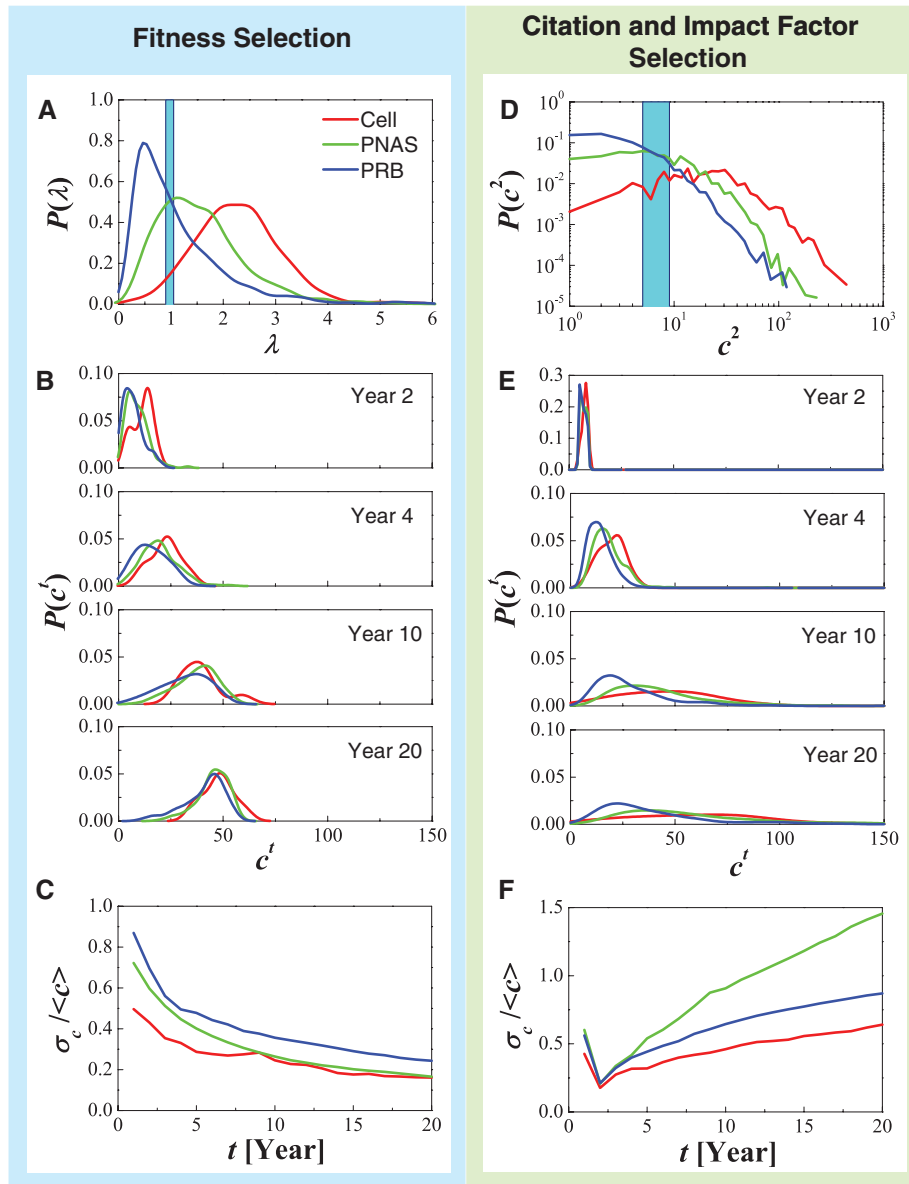


Fig. 2. Evaluating long-term impact. (A) Fitness distribution $P(\lambda)$ for papers published by *Cell*, *PNAS*, and *PRB* in 1990. Shaded area indicates papers in the $\lambda \approx 1$ range, which were selected for further study. (B) Citation distributions for papers with fitness $\lambda \approx 1$, highlighted in (A), for years 2, 4, 10, and 20 after publication. (C) Time-dependent relative variance of citations for papers selected in (A). (D) Citation distribution 2 years after publication $P(c^2)$ for papers published by *Cell*, *PNAS*, and *PRB*. Shaded area highlights papers with $c^2 \in [5, 9]$ that were selected for further study. (E) Citation distributions for papers with $c^2 \in [5, 9]$, selected in (D), after 2, 4, 10, and 20 years. (F) Time-dependent relative variance of citations for papers selected in (D).

We measured for each paper published by them the fitness λ , obtaining their distinct journal-specific $P(\lambda)$ fitness distribution (Fig. 2A). We then selected all papers with comparable fitness $\lambda \approx 1$ and followed their citation histories. As expected, they follow different paths: *Cell* papers ran slightly ahead and *PRB* papers stay behind, resulting in distinct $P(c^T)$ distributions for years $T = 2 \div 4$. Yet, by year 20 the cumulative number of citations acquired by these papers shows a notable convergence to each other (Fig. 2B), supporting our prediction that given their similar fitness λ , eventually they will have the same ultimate impact: $c^\infty = 51.5$. To quantify the magnitude of the observed convergence, we measured the coefficient of variation σ_c/c for $P(c^T)$, finding that this ratio decreases with time (Fig. 2C). This helps us move beyond visual inspection, offering quantitative evidence that in the long run the differences in citation counts between these papers vanishes with time, as predicted by our model. In contrast, if we choose all papers with the same number of citations at year two (i.e., the same c^2 , Fig. 2D), the citations acquired by them diverge with time, and σ_c/c increases (Fig. 2, E and F), supporting our conclusion that these quantities lack predictability. Therefore, λ and c^∞ offer a journal independent measure of a publication's long-term impact.

The model (Eqs. 3 to 5) also helps connect the IF, the traditional measure of impact of a scientific journal, to the journal's Λ , M , and Σ parameters

(the analogs of λ , μ , and σ ; supplementary materials S4)

$$IF \approx \frac{m}{2} \left\{ \exp \left[\Lambda \Phi \left(\frac{M_1 - M}{\Sigma} \right) \right] - \exp \left[\Lambda \Phi \left(\frac{M_2 - M}{\Sigma} \right) \right] \right\} \quad (8)$$

Knowing Λ , in analog with Eq. 6 we can calculate a journal's ultimate impact as $C^\infty = m(e^\Lambda - 1)$, representing the total number of citations a paper in the journal will receive during its lifetime. As we show in the supplementary materials S4, Eq. 8 predicts a journal's IF in good agreement with the values reported by ISI (Institute for Scientific Information). Equally important, it helps us understand the mechanisms that influence the evolution of the IF, as illustrated by the changes in the impact factor of *Cell* and *New England Journal of Medicine* (*NEJM*). In 1998, the IFs of *Cell* and *NEJM* were 38.7 and 28.7, respectively (Fig. 3A). Over the next decade, there was a remarkable reversal: *NEJM* became the first journal to reach IF = 50, whereas *Cell*'s IF decreased to around 30. This raises a puzzling question: Has the impact of papers published by the two journals changed so dramatically? To answer this, we determined Λ , M , and Σ for both journals from 1996 to 2006 (Fig. 3, D to F). Although Σ were indistinguishable (Fig. 3D), we find that the fitness of *NEJM* increased from $\Lambda = 2.4$ (1996) to $\Lambda = 3.33$ (2005), increasing the journal's ultimate impact from $C^\infty = 300$ (1996) to 812 (2005) (Fig.

3B). But *Cell*'s Λ also increased in this period (Fig. 3E), moving its ultimate impact from $C^\infty = 366$ (1996) to 573 (2005). If both journals attracted papers with increasing long-term impact, why did *Cell*'s IF drop and *NEJM*'s grow? The answer lies in changes in the impact time $T^* = \exp(M)$: Whereas *NEJM*'s impact time remained unchanged at $T^* \approx 3$ years, *Cell*'s T^* increased from $T^* = 2.4$ years to $T^* = 4$ years (Fig. 3C). Therefore, *Cell* papers have gravitated from short- to long-term impact: A typical *Cell* paper gets 50% more citations than a decade ago, but fewer of the citations come within the first 2 years (Fig. 3C, inset). In contrast, with a largely unchanged T^* , *NEJM*'s increase in Λ translated into a higher IF. These conclusions are fully supported by the $P(\lambda)$ and $P(\mu)$ distributions for individual papers published by *Cell* and *NEJM* in 1996 and 2005: Both journals show a shift to higher-fitness papers (Fig. 3G), but whereas $P(\mu)$ is largely unchanged for *NEJM*, there is a shift to higher- μ papers in *Cell* (Fig. 3H).

Can we use the developed framework to predict the future citations of a publication? For this, we adopted a framework borrowed from weather predictions and data mining: We used paper i 's citation history up to year T_{Train} after publication (training period) to estimate λ_i , μ_i , and σ_i and then used the model Eq. 3 to predict its future citations c_i^t and Eq. 6 to determine its ultimate impact c_i^∞ . The uncertainties in estimating λ_i , μ_i , and σ_i from the inherently noisy citation histories affect our

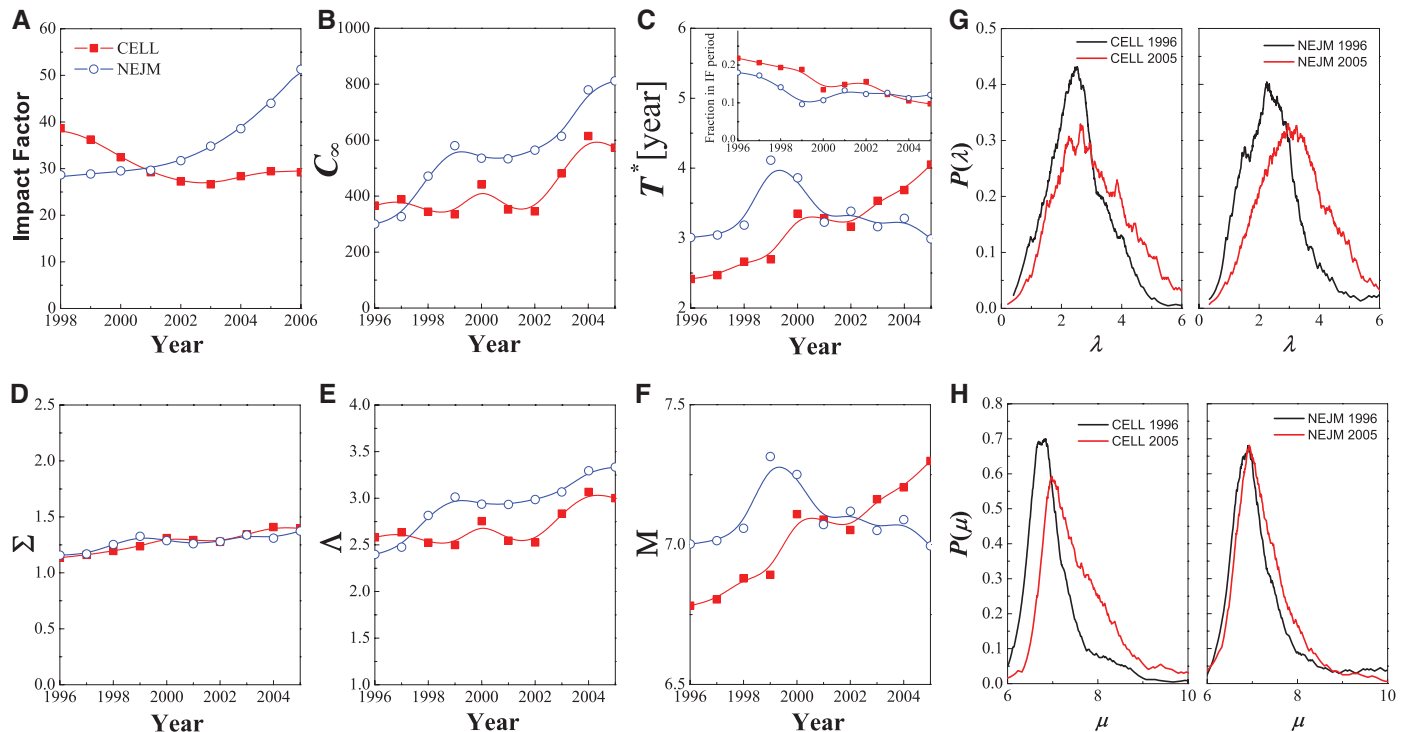


Fig. 3. Quantifying changes in a journal's long-term impact. (A) IF of *Cell* and *NEJM* reported by Thomson Reuters from 1998 to 2006. (B) Ultimate impact C^∞ (see Eq. 6) of papers published by the two journals from 1996 to 2005. (C) Impact time T^* (Eq. 7) of papers published by the two journals from 1996 to 2005. (Inset) Fraction of citations that contribute to

the IF. (D to F) The measured time-dependent longevity (Σ), fitness (Λ), and immediacy (M) for the two journals. (G) Fitness distribution for individual papers published by *Cell* (left) and *NEJM* (right) in 1996 (black) and 2005 (red). (H) Immediacy distributions for individual papers published by *Cell* (left) and *NEJM* (right) in 1996 (black) and 2005 (red).

predictive accuracy (supplementary materials S2.6). Hence, instead of simply interpolating Eq. 3 into the future, we assigned a citation envelope to each paper, explicitly quantifying the uncertainty of our predictions (supplementary materials S2.6). We show (Fig. 4A) the predicted most likely citation path (red line) with the uncertainty envelope (gray area) for three papers, based on a 5-year training period. Two of the three papers fall within the envelope; for the third, however, the model overestimated the future citations. Increasing the training period enhanced the predictive accuracy (Fig. 4B).

To quantify the model's overall predictive accuracy, we measured the fraction of papers that

fall within the envelope for all *PR* papers published in 1960s. That is, we measured the z_{30} score for each paper, capturing the number of standard deviations (z_{30}) the real citations c^{30} deviate from the most likely citation 30 years after publication. The obtained $P(z_{30})$ distribution across all papers decayed fast with z_{30} (Fig. 4C), indicating that large z values are extremely rare. With $T_{\text{Train}} = 5$, only 6.5% of the papers left the prediction envelope 30 years later; hence, the model correctly approximated the citation range for 93.5% of papers 25 years into the future.

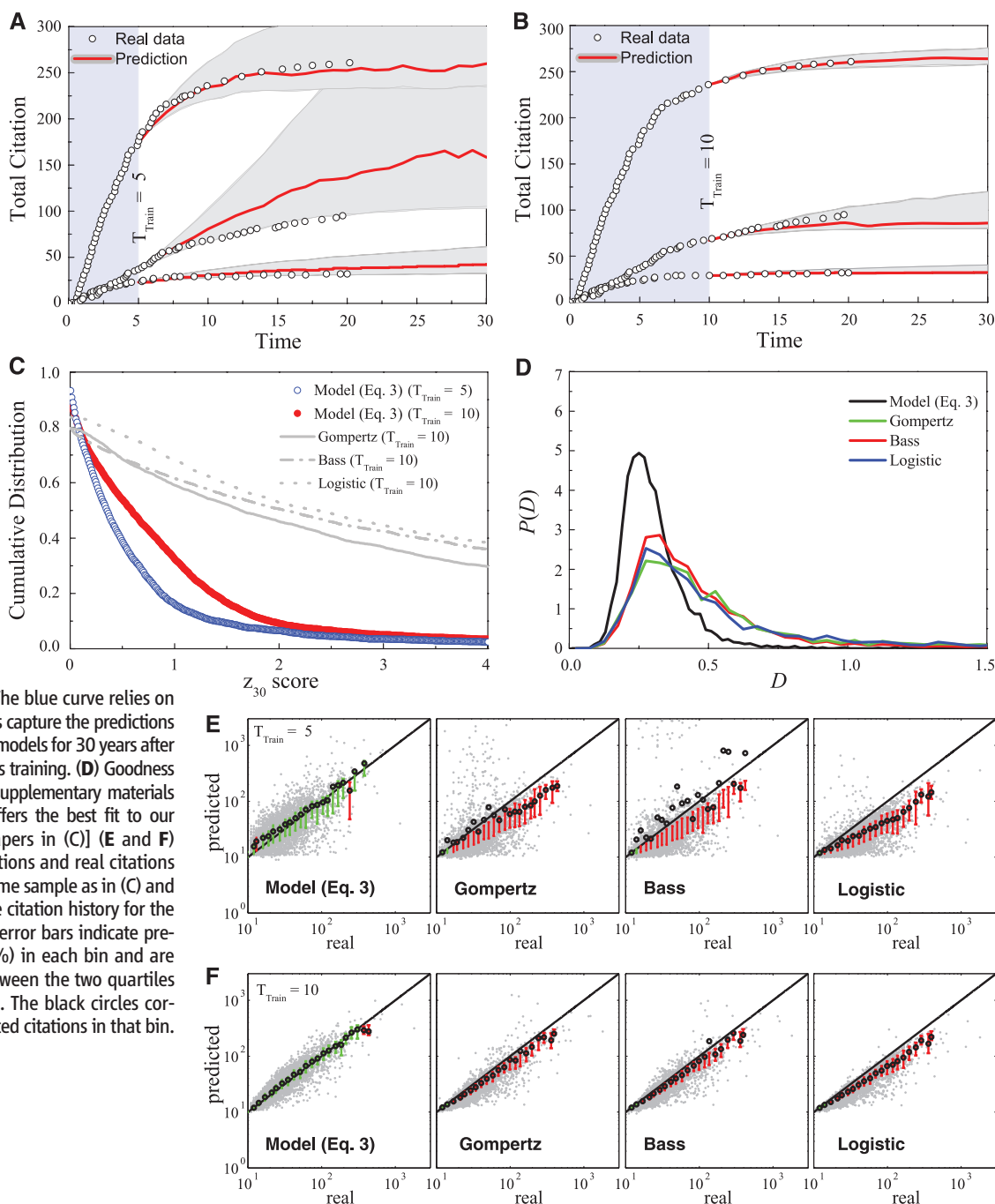
The observed accuracy prompts us to ask whether the proposed model is unique in its abil-

ity to capture future citation histories. We therefore identified several models that either have been used in the past to fit citation histories or have the potential to do so: the logistic (26), Bass (27), and Gompertz (26, 28) models (for formulae, see supplementary materials and table S2).

We fit the predictions of these models to *PR* papers and used the weighted Kolmogorov-Smirnov (KS) test to evaluate their goodness of fit (see eq. S43 for definition), capturing the maximum deviation between the fitted and the empirical data. The lowest KS distribution across most papers was observed with Eq. 3, indicative of the best fit (Fig. 4D). The reason is illustrated in fig. S18:

Fig. 4. Predicting future citations. (A and B) Prediction envelopes for three papers obtained by using 5 (A) and 10 (B) years of training

(shaded vertical area). The middle curve offers an example of a paper for which the prediction envelope misses the future evolution of the citations. Each envelope illustrates the range for which $z \leq 1$. Comparing (A) and (B) illustrates how the increasing training period decreases the uncertainty of the prediction, resulting in a narrower envelope. (C) Complementary cumulative distribution of z_{30} [$P > (z_{30})$] (see also supplementary materials S2.6). We selected papers published in 1960s in the *PR* corpus that acquired at least 10 citations in 5 years (4492 in total). The red curve captures predictions for 30 years after publication for $T_{\text{Train}} = 10$, indicating that for our model 93.5% papers have $z_{30} \leq 2$. The blue curve relies on 5-year training. The gray curves capture the predictions of Gompertz, Bass, and logistic models for 30 years after publication by using 10 years as training. (D) Goodness of fit using weighted KS test (supplementary materials S3.3), indicating that Eq. 3 offers the best fit to our testing base [same as the papers in (C)] (E and F) Scatter plots of predicted citations and real citations at year 30 for our test base [same sample as in (C) and (D)], using as training data the citation history for the first 5 (E) or 10 (F) years. The error bars indicate prediction quartiles (25 and 75%) in each bin and are colored green if $y = x$ lies between the two quartiles in that bin and red otherwise. The black circles correspond to the average predicted citations in that bin.



The symmetric $c(t)$ predicted by the logistic model cannot capture the asymmetric citation curves. Although the Gompertz and the Bass models predict asymmetric citation patterns, they also predict an exponential (Bass) or double-exponential (Gompertz) decay of citations (table S2) that is much faster than observed in real data. To quantify how these deviations affect the predictive power of each of these models, we used a 5- and a 10-year training period to fit the parameters of each model and computed the predicted most likely citations at year 30 (Fig. 4, E and F). Independent of the training period, the predictions of the logistic, Bass, and Gompertz models always lay outside the 25 to 75% prediction quartiles (red bars), systematically underestimating future citations. In contrast, the prediction of Eq. 3 for both training periods was within the 25 to 75% quantiles, its accuracy visibly improving for the 10-year training period (Fig. 4F). In supplementary materials S3.3, we offer additional quantitative assessment of these predictions (fig. S19), demonstrating our model's predictive power pertaining to both the fraction of papers whose citations it correctly predicts and the magnitude of deviations between predicted and the real citations. The predictive limitations of the current models were also captured by their $P(z_{30})$ distribution, indicating that for the logistic, Bass, and Gompertz models more than half of the papers underestimate with more than two standard deviations the true citations ($z > 2$) at year 30 (Fig. 4C), in contrast with 6.5% for the proposed model (Eq. 3).

Ignoring preferential attachment in Eq. 2 leads to the lognormal model, containing a lognormal temporal decay modulated by a single fitness parameter. As we analytically show in supplementary materials S3.4, for small fitness Eq. 3 converged to the lognormal model, which correctly captured the citation history of small impact papers. The lognormal model failed, however, to predict the citation patterns of medium- to high-impact papers (fig. S20). The proposed model therefore allows us to analytically predict the citation threshold when preferential attachment becomes relevant. The calculations indicate that the lognormal model is indistinguishable from the predictions of Eq. 3 for papers that satisfy the equation

$$\sum_{n=2}^{\infty} \frac{1}{n!} \Phi^n \lambda^n < 1 \quad (9)$$

Solving this equation predicts $\lambda < 0.25$, equivalent with the citation threshold $c^\infty < 8.5$, representing

the theoretical bound for preferential attachment to turn on. This analytical prediction is in close agreement with the empirical finding that preferential attachment is masked by initial attractiveness for papers with fewer than seven citations (29). Note that the lognormal function has been proposed before to capture the citation distribution of a body of papers (15). However, the lognormals appearing in (15) and in the lognormal model discussed above have different origins and implications (supplementary materials S2.5.2).

The proposed model has obvious limitations: It cannot account for exogenous “second acts,” like the citation bump observed for superconductivity papers after the discovery of high-temperature superconductivity in the 1980s, or delayed impact, like the explosion of citations to Erdős and Rényi's work 4 decades after their publication, following the emergence of network science (3, 20, 21, 23).

Our findings have policy implications, because current measures of citation-based impact, from IF to Hirsch index (4, 17), are frequently integrated in reward procedures, the assignment of research grants, awards, and even salaries and bonuses (30), despite their well-known lack of predictive power. In contrast with the IF and short-term citations that lack predictive power, we find that c^∞ offers a journal-independent assessment of a paper's long term impact, with a meaningful interpretation: It captures the total number of citations a paper will ever acquire or the discovery's ultimate impact. Although additional variables combined with data mining could further enhance the demonstrated predictive power, an ultimate understanding of long-term impact will benefit from a mechanistic understanding of the factors that govern the research community's response to a discovery.

References and Notes

1. E. Garfield, *JAMA* **295**, 90–93 (2006).
2. D. J. Price, *Science* **149**, 510–515 (1965).
3. S. Redner, *Phys. Today* **58**, 49 (2005).
4. J. E. Hirsch, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 16569–16572 (2005).
5. S. Lehmann, A. D. Jackson, B. E. Lautrup, *Nature* **444**, 1003–1004 (2006).
6. B. F. Jones, S. Wuchty, B. Uzzi, *Science* **322**, 1259–1262 (2008); 10.1126/science.1158357.
7. F. Radicchi, S. Fortunato, C. Castellano, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17268–17272 (2008).
8. J. A. Evans, J. Reimer, *Science* **323**, 1025 (2009).
9. J. A. Evans, J. G. Foster, *Science* **331**, 721–725 (2011).
10. A.-L. Barabási, C. Song, D. Wang, *Nature* **491**, 40 (2012).
11. L. Egghe, *Scientometrics* **69**, 131–152 (2006).
12. A. Fersht, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 6883–6884 (2009).
13. F. Radicchi, S. Fortunato, B. Markines, A. Vespignani, *Phys. Rev. E* **80**, 056103 (2009).
14. P. O. Seglen, *BMJ* **314**, 498–502 (1997).
15. M. J. Stringer, M. Sales-Pardo, L. A. Nunes Amaral, *PLoS ONE* **3**, e1683 (2008).
16. T. S. Kuhn, *The Structure of Scientific Revolutions* (University of Chicago Press, Chicago, 1996).
17. D. E. Acuna, S. Allesina, K. P. Kording, *Nature* **489**, 201–202 (2012).
18. G. J. Peterson, S. Pressé, K. A. Dill, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16023–16027 (2010).
19. S. Redner, *Eur. Phys. J. B* **4**, 131–134 (1998).
20. A.-L. Barabási, R. Albert, *Science* **286**, 509–512 (1999).
21. G. Caldarelli, *Scale-Free Networks* (Oxford Univ. Press, Oxford, 2007).
22. M. Medo, G. Cimini, S. Gualdi, *Phys. Rev. Lett.* **107**, 238701 (2011).
23. S. N. Dorogovtsev, J. F. F. Mendes, *Evolution of Networks: From Biological Nets to the Internet and WWW* (Oxford Univ. Press, Oxford, 2003).
24. G. Bianconi, A.-L. Barabási, *Europhys. Lett.* **54**, 436–442 (2001).
25. G. Caldarelli, A. Capocci, P. De Los Rios, M. A. Muñoz, *Phys. Rev. Lett.* **89**, 258702 (2002).
26. V. Mahajan, E. Muller, F. M. Bass, *J. Mark.* **54**, 1–26 (1990).
27. F. M. Bass, *Manage. Sci.* **50** (suppl.), 1833–1840 (2004).
28. B. Gompertz, *Philos. Trans. R. Soc. London* **115**, 513–583 (1825).
29. Y. H. Eom, S. Fortunato, *PLOS ONE* **6**, e24926 (2011).
30. I. Fuyuno, D. Cyranoski, *Nature* **441**, 792 (2006).

Acknowledgments: The authors thank P. Azoulay, C. Hidalgo, J. Loscalzo, D. Pedreschi, B. Uzzi, M. Vidal, and members of Center for Complex Network Research for insightful discussions and the anonymous referee for suggesting the lognormal model, which led to the analytical prediction of the citation threshold for preferential attachment. The PR data set is available upon request through American Physical Society. Supported by Lockheed Martin Corporation (SRA 11.18.11), the Network Science Collaborative Technology Alliance is sponsored by the U.S. Army Research Laboratory under agreement W911NF-09-2-0053, Defense Advanced Research Projects Agency under agreement 11645021, and the Future and Emerging Technologies Project 317 532 “Multiplex” financed by the European Commission.

Supplementary Materials

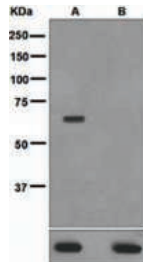
www.sciencemag.org/content/342/6154/127/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S25
Tables S1 to S4
References (31–44)

14 March 2013; accepted 4 September 2013
10.1126/science.1237825

What can **RabMAbs**[®] do for you?

#4	High Affinity
#5	High Specificity
#6	Novel Epitopes
#7	Multiple Applications

RabMAbs[®] offer high specificity which is essential in detecting post-translational modifications such as phosphorylation:



WB on NIH/3T3 using anti-Akt 1 Phospho (pT450) RabMAb (ab108266). (A) untreated or (B) treated with Lambda Phosphatase. Akt1 (ab32510) as a pan control

Akt1 RabMAb (ab32510)



Rabbit Monoclonal Antibodies (RabMAbs[®]) offer multiple advantages to bring you the highest quality antibody possible.

Discover more at abcam.com/RabMAbs





cell sciences
cytokine center



www.cellsciences.com

Buy one, get one free

Stock up now on select cytokines, growth factors and chemokines.

Order any two vials from this list for just \$235

Enter promo code CYT241 when you place your order on-line or mention the promo code to get your discount when calling our toll-free number 888-769-1246.

These recombinant proteins are low endotoxin, carrier-free, highly pure, biologically active and suitable for cell culture and animal studies. Get two of the same item or pick any two different items from the list.* See our web site for complete product specifications.

Browse our web site of recombinant proteins, including hundreds more cytokines, growth factors, chemokines and neurotrophins. Bulk quantities of these proteins are available at competitive pricing. Call or visit our web site for details. Cell Sciences also carries corresponding antibodies and ELISA kits.

Catalog No:	Item:	Size:
CRB100B	Recombinant Human BMP-2	10 µg
CRC400B	Recombinant Human CNTF	20 µg
CRF000B	Recombinant Human FGF-acidic/FGF1	50 µg
CRF001B	Recombinant Human FGF-basic/FGF2	50 µg
CRK300B	Recombinant Human FGF-7/KGF	10 µg
CRG300B	Recombinant Human G-CSF/CSF3	10 µg
CRG100B	Recombinant Human GM-CSF/CSF2	20 µg
CRG101B	Recombinant Mouse GM-CSF/CSF2	20 µg
CRG500B	Recombinant Human GRO-alpha/CXCL1	25 µg
CRG502B	Recombinant Rat GRO-alpha/KC/CXCL1	25 µg
CRI004B	Recombinant Human IFN-alpha 2b	100 µg
CRI000B	Recombinant Human IFN-gamma	100 µg
CRI001B	Recombinant Mouse IFN-gamma	100 µg
CRI002B	Recombinant Rat IFN-gamma	100 µg
CRI500B	Recombinant Human IGF-1	100 µg
CRI100B	Recombinant Human IL-2	50 µg
CRI153B	Recombinant Human IL-10	10 µg
CRI137B	Recombinant Human IL-15	10 µg
CRI162B	Recombinant Human IL-17	25 µg
CRI172B	Recombinant Human IL-21	10 µg
CRI225B	Recombinant Human IL-33	10 µg
CRM151B	Recombinant Human M-CSF/CSF1	10 µg
CRR000B	Recombinant Human RANTES/CCL5	20 µg
CRS000B	Recombinant Human SDF-1 alpha	10 µg
CRS002B	Recombinant Human SDF-1 beta	10 µg
CRT100B	Recombinant Human TNF-alpha	50 µg
CRT192B	Recombinant Mouse TNF-alpha	20 µg
CRV000B	Recombinant Human VEGF 165	10 µg
CRV014B	Recombinant Mouse VEGF 165	10 µg

* Good on orders in US and Canada only. This offer may not be combined with other offers or discounts. All products for research use only.

CELL SCIENCES INC • 480 NEPONSET STREET, BUILDING 12A, CANTON, MA 02021 • INFO@CELLSCIENCES.COM

TOLL FREE: (888) 769-1246 • TEL: (781) 828-0610 • FAX: (781) 828-0542 • WEB WWW.CELLSCIENCES.COM

Free up your time



Automated sample and assay technologies by QIAGEN

Automated solutions from sample to result:

- The widest choice of sample processing protocols
- Low-, medium-, and high-throughput automation
- Leading solutions for molecular testing
- Plug-and-play automated sample preparation
- Quantitative, real-time PCR detection
- Automated analysis of DNA fragments and RNA
- High-resolution sequence-based DNA detection and quantification

Making improvements in life possible — www.qiagen.com



Better results — on any sequencing platform

Get the most from your NGS

Discover new and innovative solutions,
dedicated for use with any NGS workflow

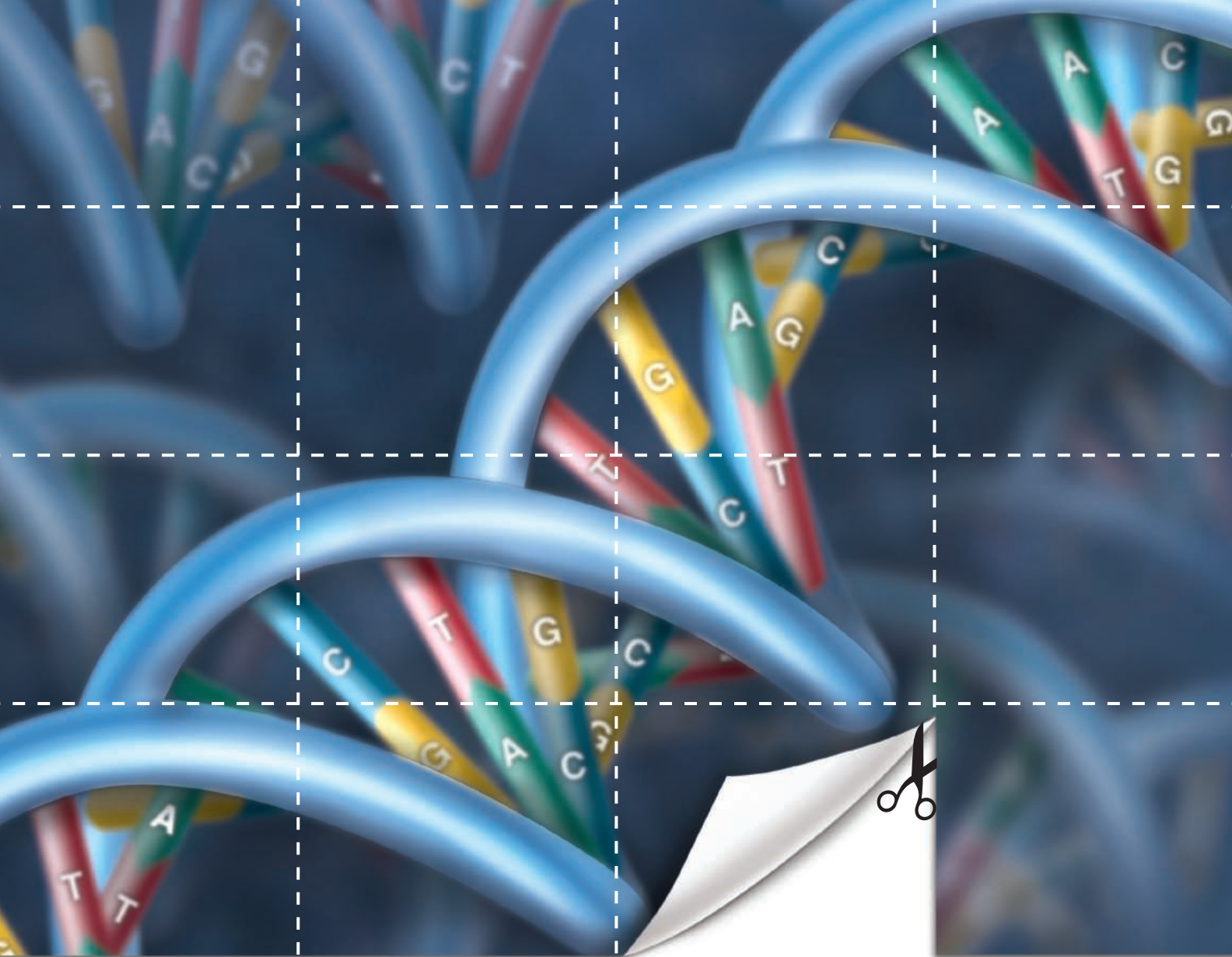


Streamline your next-generation sequencing (NGS) workflow and achieve high-quality results you can rely on.

- Highly specific and selective nucleic acid purification and target enrichment
- Unbiased whole genome amplification from a single cell
- High DNA library yields using optimized workflows that allow ~50% time-savings
- Easy-to-use, spin-column-based size selection of DNA for library construction
- Outstanding results on any sequencing platform

Visit www.qiagen.com/goto/NGS to learn more!





Cut smarter.

Restriction enzymes from NEB –
now with CutSmart™ Buffer

You make smart choices every day. Why stop there? The choice to use restriction enzymes from New England Biolabs is now even easier.

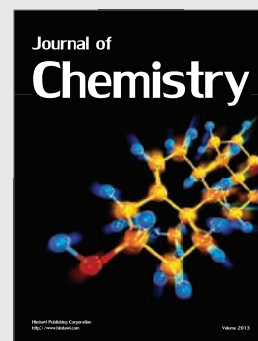
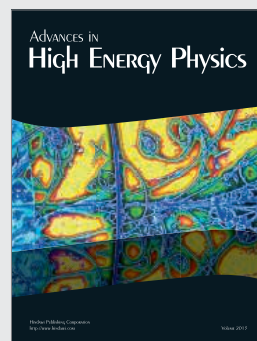
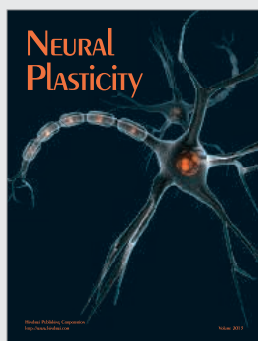
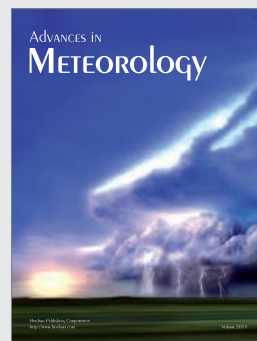
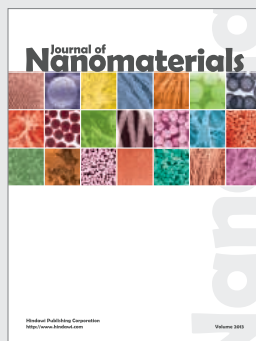
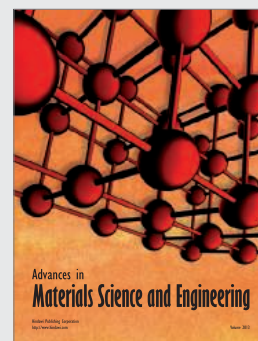
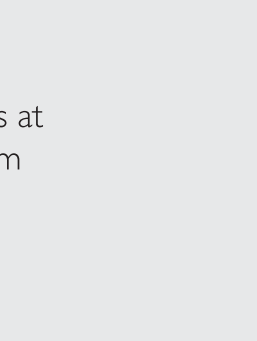
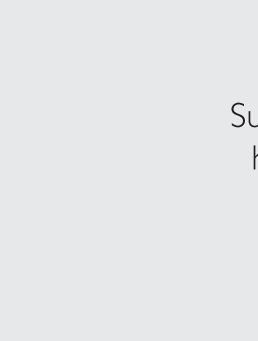
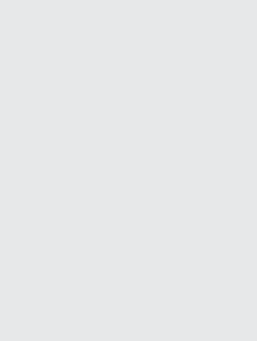
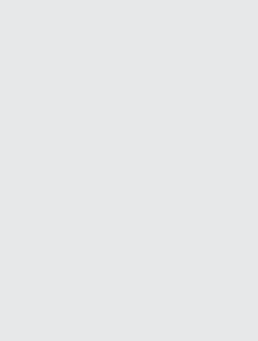
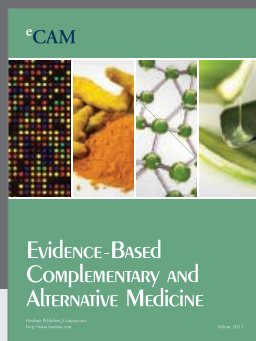
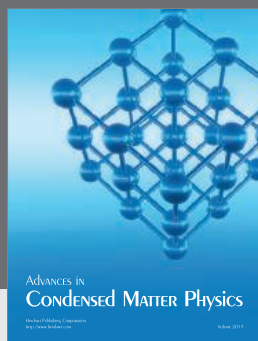
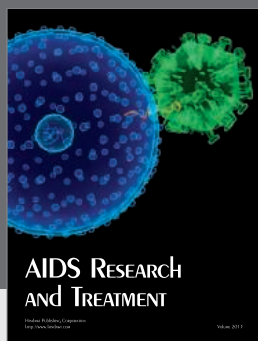
- Choose from > 200 restriction enzymes supplied with a single buffer
- Simplify your double digest reactions
- Reduce your pipetting steps by no longer having to add BSA

Now, that's Smart!



Explore the smarter choice at
NEBCutSmart.com

 NEW ENGLAND
BioLabs® Inc.
enabling technologies in the life sciences



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

SimpleStep[™] ELISA kits – make your life easier



Mix → Wash → Read. It's that easy.

Discover our new single wash sandwich ELISA assay offering improved performance.

Discover more at [abcam.com/SimpleStep](https://www.abcam.com/SimpleStep)

 **SimpleStep[™]**

BD Accuri™ C6 Systems

Perfectly suited to support demanding educational environments.



Learn. Teach. Master.

The BD Accuri C6 is a perfect fit for personal research, student mastery, and core lab use. It's easy to learn and operate, powerful enough for the majority of routine flow applications, and small enough for easy transport, making it perfect for the demands of the educational environment. Affordably priced, it may just be one of the best valued instrument in your lab.

The 4-color BD Accuri C6 allows for teaching and learning in a guided way, through templates, and factory pre-sets. The system also collects and stores 7.2 decades of data to empower novice users to make corrections if errors are made.



Helping all people
live healthy lives

Those new to flow cytometry will appreciate the simplicity of the BD Accuri C6, while experts will appreciate the extensive capabilities and workflow advantages. Core lab managers will be able to better allocate resources, offloading routine applications and reserving higher-end systems for more complex, multi-parameter duties. Researchers will find it empowering for personal research and affordable.

Find out how your teaching institution and lab could benefit from owning BD Accuri C6 systems by visiting bdbiosciences.com/go/learn.

Call for 2013 Cozzarelli Prize Nominations

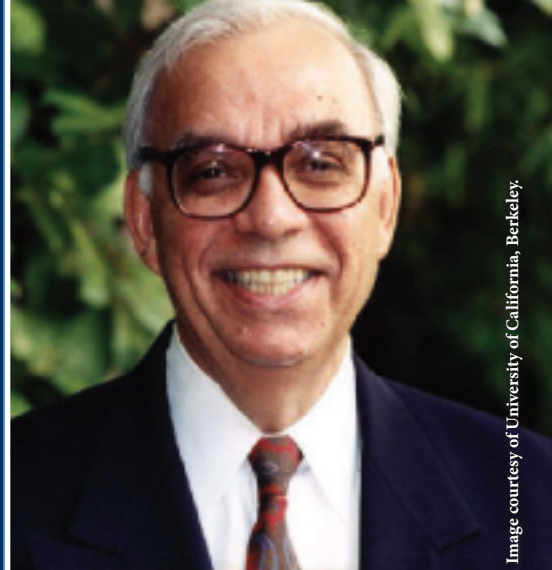
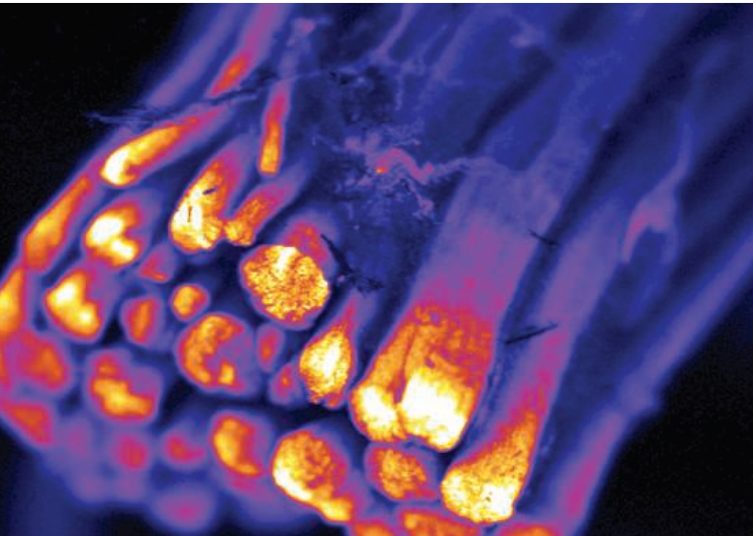


Image courtesy of University of California, Berkeley.

Nicholas R. Cozzarelli, former PNAS Editor-in-Chief



The PNAS Editorial Board is now accepting nominations through January 10, 2014 for the 2013 Cozzarelli Prize. This award recognizes scientific excellence and will be given to six papers published in PNAS during 2013.

Nominations should be sent to pnas@nas.edu and should include a citation and brief explanation of the merits of the work. The award recipients will be recognized during the PNAS Editorial Board Meeting and the NAS Annual Meeting Awards Ceremony on April 27, 2014 in Washington, DC.

AAAS|2014 ANNUAL MEETING

13-17 FEBRUARY • CHICAGO

MEETING GLOBAL CHALLENGES:
DISCOVERY AND INNOVATION

The 2014 AAAS Annual Meeting features several opportunities to help scientists tell their stories.

- Plenary lecture by Alan Alda, “Getting Beyond a Blind Date with Science”
 - Communicating Science seminar with sessions on engaging with journalists, social media, and public events
 - Symposia in the Communication and Public Programs track, for example:
 - Improvisation for Scientists: Making a Human Connection
 - What Do People Think about Science and Technology?
U.S. and International Public Opinion
 - Where’s My Flying Car? Science, Science Fiction, and a Changing Vision of the Future
- and much more!

The program schedule is now available online.

Register today!

www.aaas.org/meetings

 #AAASmtg



Auto XY Stage

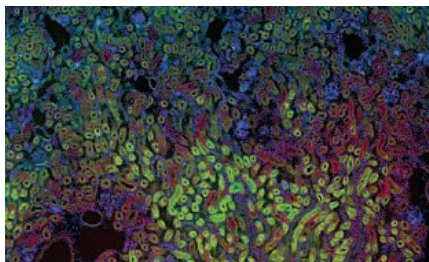
Auto Focus

Auto Cell Counting

Auto Image Capture



AutoEasy



High quality, automated imaging made simple.

With so many ways to automate cell counting and image analyses, the Cytation™3 Cell Imaging Multi-Mode Reader saves time and improves your workflow; all at a reasonable price. It can also be upgraded at any time to a Hybrid multi-mode microplate reader for enhanced flexibility and value.

To see all it can do for live cell applications, visit www.cytation3.com.

Think Possible

BioTek®



www.biotek.com



Dr. Chinyere K. Okoro, Branco Weiss fellow since 2013

Know what science will look like tomorrow? Apply today.

The magnitude of challenges we face today requires people with fresh thinking and novel approaches. To help find new ways forward, Society in Science - The Branco Weiss Fellowship gives extraordinary postdocs and engineers a generous grant to pursue an unconventional project for up to five years anywhere in the world. Have an idea that could change tomorrow? Get in touch with us today!

www.society-in-science.org

society
in science
The Branco Weiss Fellowship

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

LAMBDA VF-5 Tunable filter changer

NEW!



Introducing the world's first filter changer to use tunable thin-film optical filters. The Sutter **LAMBDA VF-5** allows you to quickly access any center bandpass from 330 to 800nm in nanometer increments. Building on the VersaChrome® filters from Semrock®, the **LAMBDA VF-5** maintains transmission over the tuning range of each filter.

Easy Wavelength Selection

Wavelength range as wide as 330-800nm
Keypad or computer interface (USB or serial)

Flexible

Suitable for excitation or emission
Easily switch between fluorophore combinations
Optional liquid light guide offers absolute vibration isolation
Images pass through filters

Thin filter advantage

High transmission
Steep spectral edges
High out-of-band blocking
Polarization independence
(s and p nearly identical)

SUTTER INSTRUMENT

PHONE: 415.883.0128 | FAX: 415.883.0572
EMAIL: INFO@SUTTER.COM | WWW.SUTTER.COM

Redefining the Link Between Basic and Applied Science



The Graduate School of Biomedical Sciences

at the Icahn School of Medicine at Mount Sinai in New York City offers PhD and MD/PhD students the opportunity to earn their degrees in Biomedical Sciences or Neurosciences in one of the most innovative environments in the country.



- **Ranked #2** by *U.S. News & World Report* among the 30 freestanding medical schools with an associated graduate school
- **Ranked #5** in the nation for National Institutes of Health funding per investigator
- The Graduate School's faculty are part of more than 200 diverse research laboratories

The Graduate School of Biomedical Sciences

Icahn School of Medicine at Mount Sinai

FIND OUT MORE:

www.ichn.mssm.edu/gradschool

EMAIL: grads@mssm.edu



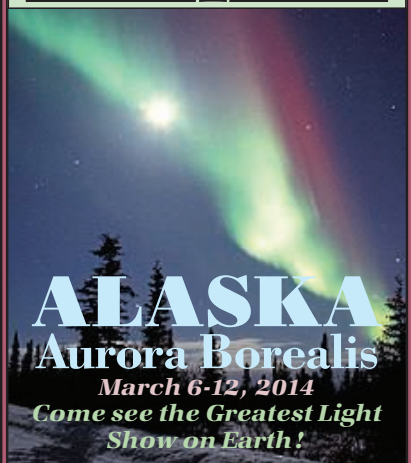
Join the Conversation!

Twitter is a great way to connect with AAAS members and staff about the issues that matter to you most. Be a part of the discussion while staying up-to-date on the latest news and information about your personal member benefits.

Follow us @AAASmember
and join the conversation
with #AAAS



AAAS Travels



ALASKA Aurora Borealis

March 6-12, 2014

Come see the Greatest Light
Show on Earth!

See the dazzling night sky, spectacular snow-covered mountain peaks, grizzlies, moose, bald eagles, Denali (Mt. McKinley), and the famed Aurora Borealis (Northern Lights), the greatest light show on Earth! \$2,795 + air.

For a detailed brochure,
please call (800) 252-4910

All prices are per person twin share + air



BETCHART EXPEDITIONS inc.
17050 Montebello Rd, Cupertino, CA 95014
Email: AAASInfo@betchartexpeditions.com
www.betchartexpeditions.com

CHEMBRIDGE: A MAJOR FORCE IN BUILDING BLOCKS

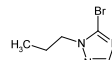
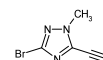
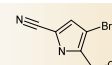
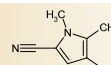
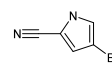
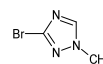
Over 14,000 unique reagents
for medicinal chemistry
and library synthesis

Available overnight from
San Diego stock via our
e-commerce portal
Hit2Lead.com

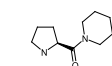
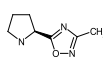
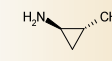
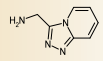
AMINO ACIDS



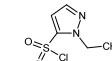
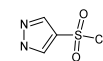
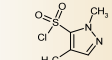
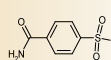
ARYL BROMIDES



ALIPHATIC AMINES



SULFONYL HALIDES



STAY INFORMED! STAY CONNECTED!

Get more from your
AAAS membership



Are you currently registered to receive e-mails from AAAS and *Science*?

E-mail is the primary way that AAAS communicates with our members about AAAS programs, new member benefits, invitations to special events, and, of course, the latest news and research being published in *Science*.

Sign up today to receive e-mails from AAAS and ensure that you are getting the most out of your membership and *Science* subscription.*

To get started visit: promo.aaas.org/stayconnected You'll need your AAAS Member number. Find it above your name on your *Science* mailing label.

Don't miss a thing. Sign up for e-mail communications from AAAS today!



*AAAS follows CAN-SPAM and European Safe Harbor guidelines for protecting your privacy. We will never sell your e-mail address and you can opt-out of receiving e-mails at any time.

- Available from stock in San Diego for immediate delivery
- 24-48 hour delivery for rush orders. One week delivery worldwide
- Over 95% purity by NMR & LCMS
- Availability and pricing online at www.hit2lead.com



11199 Sorrento Valley Rd., Suite 206
San Diego, CA 92121
Tel: +1-858-451-7400, Option #4
Fax: +1-858-451-7401
support@chembridge.com

www.hit2lead.com

2014 **MRS**
SPRING MEETING & EXHIBIT
April 21-25, San Francisco, CA



CALL FOR PAPERS

Abstract Deadline • November 1, 2013
Abstract Submission Site Opens • October 1, 2013

ENERGY

- A Film-Silicon Science and Technology
- B Organic and Inorganic Materials for Dye-Sensitized Solar Cells
- C Synthesis and Processing of Organic and Polymeric Materials for Semiconductor Applications
- D Materials for Photoelectrochemical and Photocatalytic Solar-Energy Harvesting and Storage
- E Earth-Abundant Inorganic Solar-Energy Conversion
- F Controlling the Interaction between Light and Semiconductor Nanostructures for Energy Applications
- G Photoactivated Chemical and Biochemical Processes on Semiconductor Surfaces
- H Defect Engineering in Thin-Film Photovoltaic Materials
- I Materials for Carbon Capture
- J Physics of Oxide Thin Films and Heterostructures
- K Nanostructures, Thin Films and Bulk Oxides—Synthesis, Characterization and Applications
- L Materials and Interfaces in Solid Oxide Fuel Cells
- M Fuel Cells, Electrolyzers and Other Electrochemical Energy Systems
- N Research Frontiers on Electrochemical Energy Storage Materials—Design, Synthesis, Characterization and Modeling
- O Novel Energy-Storage Technologies beyond Li-ion Batteries—From Materials Design to System Integration
- P Mechanics of Energy Storage and Conversion—Batteries, Thermoelectrics and Fuel Cells
- Q Materials, Technologies and Sensor Concepts for Advanced Battery Management Systems
- R Materials Challenges and Integration Strategies for Flexible Energy Devices and Systems
- S Actinides—Basic Science, Applications and Technology
- T Superconductor Materials—From Basic Science to Novel Technology

SOFT AND BIOMATERIALS

- U Soft Nanomaterials
- V Micro- and Nanofluidic Systems for Materials Synthesis, Device Assembly and Bioanalysis
- W Functional Biomaterials for Regenerative Engineering
- Y Biomaterials for Biomolecule Delivery and Understanding Cell-Niche Interactions
- Z Bioelectronics—Materials, Processes and Applications
- AA Advanced Multifunctional Biomaterials for Neuroprosthetic Interfaces

ELECTRONICS AND PHOTONICS

- BB Materials for End-of-Roadmap Devices in Logic, Power and Memory
- CC New Materials and Processes for Interconnects, Novel Memory and Advanced Display Technologies
- DD Silicon Carbide—Materials, Processing and Devices
- EE Advances in Inorganic Semiconductor Nanoparticles and Their Applications
- FF The Grand Challenges in Organic Electronics
- GG Few-Dopant Semiconductor Optoelectronics
- HH Phase-Change Materials for Memory, Reconfigurable Electronics and Cognitive Applications
- II Emerging Nanophotonic Materials and Devices
- JJ Materials and Processes for Nonlinear Optics
- KK Resonant Optics—Fundamentals and Applications
- LL Transparent Electrodes

NANOMATERIALS

- MM Nanotubes and Related Nanostructures
- NN 2D Materials and Devices beyond Graphene
- OO *De Novo* Graphene
- PP Nanodiamonds—Fundamentals and Applications
- QQ Computationally Enabled Discoveries in Synthesis, Structure and Properties of Nanoscale Materials
- RR Solution Synthesis of Inorganic Functional Materials
- SS Nanocrystal Growth via Oriented Attachment and Mesocrystal Formation
- TT Mesoscale Self-Assembly of Nanoparticles—Manufacturing, Functionalization, Assembly and Integration
- UU Semiconductor Nanowires—Synthesis, Properties and Applications
- VV Magnetic Nanomaterials and Nanostructures

GENERAL—THEORY AND CHARACTERIZATION

- WW Materials by Design—Merging Advanced *In-situ* Characterization with Predictive Simulation
- XX Shape Programmable Materials
- YY Meeting the Challenges of Understanding and Visualizing Mesoscale Phenomena
- ZZ Advanced Characterization Techniques for Ion-Beam-Induced Effects in Materials
- AAA Applications of *In-situ* Synchrotron Radiation Techniques in Nanomaterials Research
- BBB Advances in Scanning Probe Microscopy for Material Properties
- CCC *In-situ* Characterization of Material Synthesis and Properties at the Nanoscale with TEM
- DDD Atomic-Resolution Analytical Electron Microscopy of Disruptive and Energy-Related Materials
- EEE Materials Behavior under Extreme Irradiation, Stress or Temperature

SPECIAL SYMPOSIUM

- FFF Educating and Mentoring Young Materials Scientists for Career Development

www.mrs.org/spring2014

Meeting Chairs

Jose A. Garrido, Technische Universität München
Sergei V. Kalinin, Oak Ridge National Laboratory
Edson R. Leite, Federal University of Sao Carlos
David Parrillo, The Dow Chemical Company
Molly Stevens, Imperial College London

Don't Miss These Future MRS Meetings!

2014 MRS Fall Meeting & Exhibit
November 30-December 5, 2014

Hynes Convention Center & Sheraton Boston Hotel
Boston, Massachusetts

2015 MRS Spring Meeting & Exhibit
April 6-10, 2015

Moscone West & San Francisco Marriott Marquis
San Francisco, California



MATERIALS RESEARCH SOCIETY
Advancing materials. Improving the quality of life.

506 Keystone Drive • Warrendale, PA 15086-7573
Tel 724.779.3003 • Fax 724.779.8313
info@mrs.org • www.mrs.org

SCIENTIFIC CAMERA SYSTEM

The new high-speed SPOT Insight Gigabit Camera comes equipped with 4 megapixel resolution and a very large field of view that creates impressive live microscopy presentations for teaching and conferences. Designed for remarkable quality images, the SPOT Insight Gigabit Camera uses a Kodak 21.4 mm scientific CCD sensor. Cellular structures have never looked brighter or clearer. Fixed images can be captured in all common formats for easy addition to tumor board presentations or journal submissions. Low-noise circuitry enables 14 frames per second live imaging with incredible clarity that captures attention. The SPOT Insight Gigabit Camera adapts to any microscope. The easy-to-use SPOT Basic Software interface controls the camera on PCs or Macs, providing a smooth live view of the microscope slides, even during zooming and focusing. The microscope view is impressive when projected on large screens with superior color reproduction and detail.

Spot Imaging Solutions

For info: 586-731-6000 | www.spotimaging.com/gig



LIVE CELL IMAGING INCUBATION SYSTEM

Designed with the complete workflow in mind, the cellVivo incubation system is designed for precise and ergonomic environmental control of advanced live cell imaging. Following the modular approach of the IX3 range of inverted microscope frames, cellVivo is based on a “one-size-fits-all” concept, with adaptors for various IX73 and IX83 frame types, circumventing the need for multiple incubation systems for distinct microscope configurations. For ease of use and flexibility, the incubator systems can be rapidly and simply assembled, modified, and detached without the use of tools. Various “enclosure” types which act as the base incubator box, are available in a range of models, from a high-end light-shielded enclosure with incorporated safely laser-lock, to a more standard plain transparent enclosure. The intelligent light-shielded enclosures eliminate the need of a dedicated darkroom, with cell-friendly adjustable LED interior lighting and a non-reflective glass viewing window.

Olympus

For info: +49-40-23773-5913 | www.microscopy.olympus.eu

MICROSCOPE STAGE

The innovative design of the motorized high-precision microscope stage, the H01F Flat Top Stage, for upright microscopes incorporates a completely flat top plate, which eliminates any obstacle to objective rotation while ultralow profile sample holders facilitate the use of high NA objectives. High-resolution embedded *x* and *y* axis encoders provide closed loop control and the latest in miniature high torque motors allow for easy access to the condenser and other microscope adjustments. The ample top plate area facilitates the use of environmental chambers, micromanipulators, and other stage accessories. Externally adjustable limit switches allow for simple and quick stage travel adjustment making the H101F the most versatile and accurate high-precision flat top stage on the market today.

Prior Scientific

For info: 781-878-8442 | www.prior.com

COOLING STAGE

The Coolstage is a Peltier-driven SEM cooling stage for scanning electron microscopy (SEM), low vacuum, or variable pressure applications. The stage can be cooled to sub-zero temperatures for specimens that may be sensitive at ambient temperature, subject to beam damage, or may otherwise ‘sublime’ (lose water) at ambient temperatures. There are three versions of Coolstage to cover differing specimen requirements: Standard, Enhanced, and Ultra. The Standard Coolstage features include a temperature range of -30°C to +50°C at 300 Pa, self-contained cooling, temperature accuracy of +/- 1.5°C or 2% (whichever is greater), minimal image drift, cooling and heating rates of up to 30°C per minute, and keypad control with simultaneous display of actual and target temperature. The Enhanced Coolstage features a temperature range -30°C to +160°C at 300 Pa, and the Ultra Coolstage features a temperature range -50°C to +50°C at 300 Pa in addition to the specifications for the Standard Coolstage.

Electron Microscopy Sciences

For info: 215-412-8400 | www.emsdiasum.com

EMISSION SCANNING ELECTRON MICROSCOPE

The new Crossbeam series system features high speed in materials analysis and processing and a wide diversity of applications. Time intensive 3-D experiments that used to run for several days can now be completed overnight. The newly developed focused ion beam column enables fast and precise materials processing that can be observed with the field emission scanning electron microscope in real-time. High resolution over the entire voltage and current range allows users to work quickly and precisely. Designed for stability, the system ensures reproducible results even in long-term experiments. The field of application is expanded by the optionally available Massive Ablation Laser that rapidly prepares samples to access deeply buried regions of interest. Crossbeam is suitable for use in both materials and life sciences. Bioscientists can use Crossbeam, above all, for fast tomography series with high *z* resolution in cell and tissue biology.

Carl Zeiss

For info: +49-3641-64-3949 | www.zeiss.de

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by Science or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

WEBINAR

The Personal Side of Sequencing

Exploring the Role of Cancer Gene Panels and NGS in Clinical Research

Wednesday, October 23, 2013

12 noon Eastern, 9 a.m. Pacific, 5 p.m. UK, 6 p.m. Central Europe

Genetic testing methods for cancer research play an important role detecting gene variance in disease. As researchers learn more about the mechanisms of cancer, the development of accurate and affordable technologies for effective and quick detection becomes paramount. Advances in next generation sequencing (NGS) have allowed accurate variant detection that can uncover the specific genetic mutations that may drive cancer. The application of NGS to breast cancer research has enabled the development of cost-effective, multigene sequencing panels that have advanced our understanding of the disease and may in the future translate into better diagnoses and outcomes for patients. The audience will learn about the current state of breast cancer research, how data generated by NGS gene panels target variants of interest and have been developed and used in routine laboratory research, and the broader issues of breast cancer education, awareness, and community services.

Speakers



José Luis Costa, Ph.D.

IPATIMUP

Porto, Portugal



Harriet Feilotter, Ph.D.

Queen's University

Kingston, Ontario, Canada



Sandra Balladares, Ph.D.

Life Technologies

Mexico City, Mexico

REGISTER NOW!

webinar.sciencemag.org

During the webinar, the speakers will:

- Describe their clinical research into genes associated with breast cancer
- Address criteria for laboratory NGS gene panel testing
- Discuss the current landscape regarding breast cancer awareness, and the availability and affordability of research testing
- Answer your questions live on air!

Webinar sponsored by

ion torrent
by *life* technologies™

Brought to you by the
**Science/AAAS Custom
Publishing Office**



UNRAVEL DISEASE-CAUSING VARIANTS IN YOUR RESEARCH

ion torrent
Sequencing for all™

Introducing the new Ion AmpliSeq™ Exome Kit for the Ion Proton™ System

Now, more research laboratories can adopt the power of exome sequencing to identify relevant variants in rare and complex disease research faster—with increased throughput, high accuracy, and the simplest workflow from sample preparation to data analysis.

- Simplify your exome enrichment
- Sequence exomes in your lab
- Easily identify relevant variants



Learn more about exome sequencing at
lifetechnologies.com/ionexome

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.
©2013 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation and/or its affiliate(s) or their respective owners. C0114776 0813

life
technologies™

There's only one

Science



Science Careers Advertising

For full advertising details, go to ScienceCareers.org and click For Employers, or call one of our representatives.

Tracy Holmes

Worldwide Associate Director
Science Careers
Phone: +44 (0) 1223 326525

THE AMERICAS

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

Tina Burks

East Coast/West Coast/South America
Phone: 202-326-6577

Marci Gallun

Midwest/Canada
Phone: 202-326-6582

Candice Nulsen

Corporate
Phone: 202-256-1528

Online Job Posting Questions

Phone: 202-312-6375

EUROPE / INDIA / AUSTRALIA / NEW ZEALAND / REST OF WORLD

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223 326532

Axel Gesatzki

Phone: +44 (0)1223 326529

Sarah Lelarge

Phone: +44 (0) 1223 326527

Kelly Grace

Phone: +44 (0) 1223 326528

JAPAN

Yuri Kobayashi

Phone: +81-(0)90-9110-1719
E-mail: ykobayas@aaas.org

CHINA / KOREA / SINGAPORE / TAIWAN / THAILAND

Ruolei Wu

Phone: +86-1367-1015-294
E-mail: rwu@aaas.org

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. Science reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. Science encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.

Science Careers

From the journal Science



ScienceCareers.org

MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

2014 POSTDOCTORAL FELLOWSHIP PROGRAM

Applications for the postdoctoral fellowship program at the Monterey Bay Aquarium Research Institute (MBARI) are currently being accepted. MBARI is dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. Ongoing programs in marine robotics, ocean physics, chemistry, geology, and biology as well as information management and ocean instrumentation research and development exist at MBARI. Located in Moss Landing, California at the head of Monterey Bay, MBARI enjoys convenient access to diverse oceanographic environments. The institute operates research vessels equipped with remotely operated vehicles, autonomous underwater vehicles, and diverse oceanographic equipment. In addition, MBARI operates the MARS seafloor cabled observatory. MBARI is a non-profit oceanographic research institute supported by the David and Lucile Packard Foundation.

Offers will be made to candidates from the fields of biological, chemical, physical oceanography, marine geology, and ocean engineering. Candidates must be awarded the Ph.D. degree prior to commencing the two-year appointment and start during the 2014 calendar year. Applicants are encouraged to communicate with potential research sponsors at MBARI for guidance on project feasibility, relevance to ongoing research projects, and resource availability (http://www.mbari.org/about/postdoc_mentors.htm).

Application deadline: Wednesday, December 11, 2013

Selected candidates will be contacted in early March 2014.

Application requirements:

1. Curriculum vitae
2. At least three professional letters of recommendation
3. Succinct statement of the applicant's doctoral research
4. Potential research goals at MBARI
5. Supplemental Information online form (http://www.mbari.org/oed/jobs/forms/postdoc_form_2014.htm)

Competitive compensation and benefits package.

MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, disability, or veteran status.

Address your application materials to:

MBARI, Human Resources
Job code: Postdocs-2014
7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to: jobs_postdocs@mbari.org (preferred),
by mail, or fax to (831) 775-1620.



EOE • MBARI Welcomes Diversity



University of California
San Francisco

advancing health worldwide™

ASSISTANT/ASSOCIATE/ FULL PROFESSOR

Division of Craniofacial Anomalies

The Division of Craniofacial Anomalies in the Department of Orofacial Sciences, School of Dentistry, seeks to hire a faculty member at Assistant, Associate or Full Professor rank. Candidates are expected to establish a dynamic research program and to contribute to teaching and training programs.

We are interested in clinician-scientists and basic research scientists who work in one of the following areas: craniofacial development, craniofacial stem cells or tissue engineering, or genetics of craniofacial disease. Candidates should hold a Ph.D. or equivalent, M.D., or D.D.S. degree, or some combination thereof.

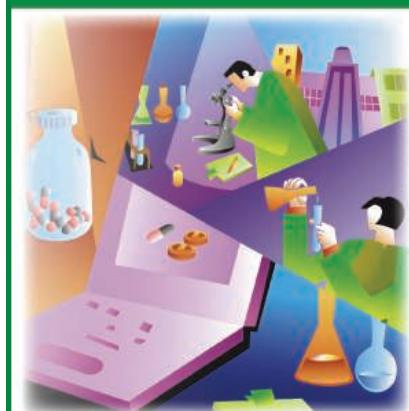
Please submit applications at:

<http://apptrkr.com/388966>

UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence. UCSF is an Affirmative Action/Equal Opportunity Employer.

CAREER TRENDS

Running
Your Lab



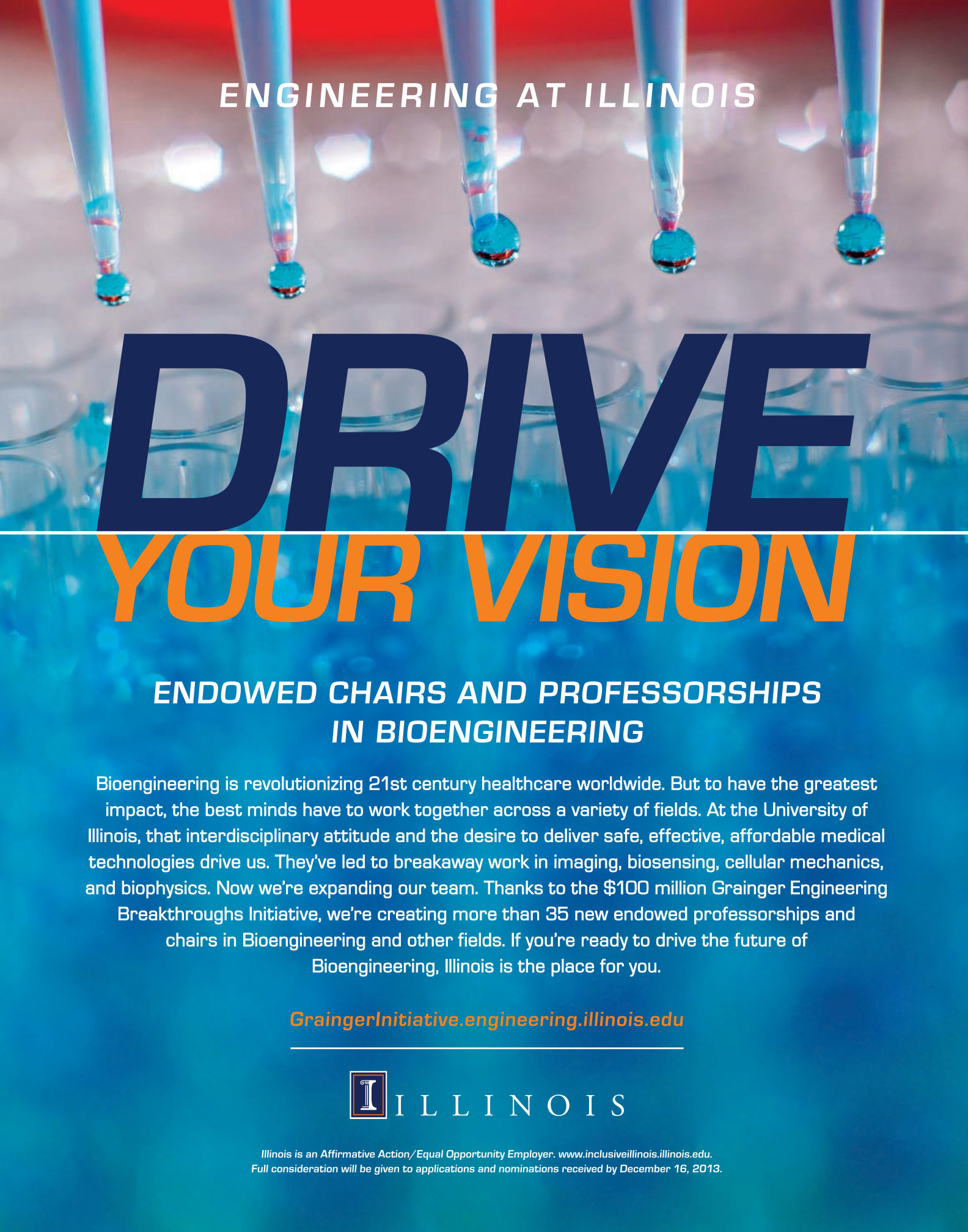
Download your free copy today at
ScienceCareers.org/booklets

Science Careers

From the journal Science



Brought to you by the
AAAS/Science Business Office



ENGINEERING AT ILLINOIS

DRIVE YOUR VISION

ENDOWED CHAIRS AND PROFESSORSHIPS IN BIOENGINEERING

Bioengineering is revolutionizing 21st century healthcare worldwide. But to have the greatest impact, the best minds have to work together across a variety of fields. At the University of Illinois, that interdisciplinary attitude and the desire to deliver safe, effective, affordable medical technologies drive us. They've led to breakaway work in imaging, biosensing, cellular mechanics, and biophysics. Now we're expanding our team. Thanks to the \$100 million Grainger Engineering Breakthroughs Initiative, we're creating more than 35 new endowed professorships and chairs in Bioengineering and other fields. If you're ready to drive the future of Bioengineering, Illinois is the place for you.

GraingerInitiative.engineering.illinois.edu



Illinois is an Affirmative Action/Equal Opportunity Employer. www.inclusiveillinois.illinois.edu.
Full consideration will be given to applications and nominations received by December 16, 2013.



University of Toronto
25 Harbord Street, Toronto, ON Canada M5S 3G5
Fax (416) 978-8532 www.csb.utoronto.ca

Cell Biology – University of Toronto

The Department of Cell & Systems Biology at the University of Toronto invites applications for a tenure-stream appointment at the rank of Assistant Professor beginning July 1, 2014.

We are seeking applicants with demonstrated excellence in research areas that will complement the strengths of the Department of Cell & Systems Biology in genomics, plant and microbial biology, cell and developmental biology, or neurosciences. We will consider all outstanding applicants but are particularly interested in candidates that utilize advanced cell biological and imaging tools for the study of whole organism model systems. The University of Toronto and its affiliated institutions represent an outstanding world-class scientific environment with a highly interactive community of researchers.

The successful candidate should have substantial postdoctoral experience and will pursue a vigorous internationally-recognized research program. In addition, we expect a strong commitment to excellence in teaching and mentoring of undergraduate and graduate students. Salary will be commensurate with qualifications and experience.

All qualified candidates are invited to apply by following the link below. Applications should include a cover letter, curriculum vitae, teaching dossier (including a statement of teaching philosophy), and a statement outlining current and future research interests. If you have questions about the position, please contact csbsearch@utoronto.ca. All application materials should be submitted online.

Please see submission guidelines at <http://uoft.me/how-to-apply>. Application materials should be combined into a single PDF file of less than 5 Mb. Applicants should also ask at least three referees to send letters directly to the department via e-mail to csbsearch@utoronto.ca by the closing date, **November 15, 2013**.

For more information about the Department of Cell & Systems Biology, please visit: <http://www.csb.utoronto.ca/>.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to the further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.



Jefferson Science Fellowship



The National Academies is pleased to announce a call for nominations and applications for the 2014 Jefferson Science Fellows program. Initiated by the Secretary of State in 2003, this fellowship program engages the American academic science, technology, engineering and medical communities in the design and implementation of U.S. foreign policy.

Jefferson Science Fellows (JSF) spend one year at the U.S. Department of State or the U.S. Agency for International Development (USAID) for an on-site assignment in Washington, D.C. that may also involve extended stays at U.S. foreign embassies and/or missions.

The fellowship is open to tenured, or similarly ranked, academic scientists, engineers and physicians from U.S. institutions of higher learning. Nominees/applicants must hold U.S. citizenship and will be required to obtain a security clearance.

The deadline for 2014-2015 program year applications/nominations is **January 13, 2014**. To learn more about the Jefferson Science Fellowship and to apply, visit the website at:

www.nas.edu/jsf

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine



EMORY

YERKES
NATIONAL
PRIMATE
RESEARCH
CENTER

DIRECTOR

YERKES NATIONAL PRIMATE RESEARCH CENTER
ROBERT W. WOODRUFF HEALTH SCIENCES CENTER

Robert W. Woodruff Health Sciences Center at Emory University seeks applications and nominations for the position of Director, Yerkes National Primate Research Center (Yerkes).

Yerkes is one of eight National Primate Research Centers sponsored by the National Center for Research Resources (NCRR) of the National Institutes of Health (NIH). The Yerkes Center was the first scientific institute in the United States dedicated to the study of nonhuman primates in behavioral and biomedical research and became a part of Emory University in 1956.

Since its founding, Yerkes has been dedicated to conducting essential basic science and translational research to advance scientific understanding and to improve the health and well-being of humans and nonhuman primates. Supported by \$67 million in research funding in fiscal year 2012, Yerkes is making landmark discoveries in vaccine development for infectious and noninfectious diseases, transplantation medicine, treatments for neurodegenerative diseases and drug addiction, and the evolutionary links between biology and behavior. The Center has been fully accredited since 1985 by the Association for Assessment and Accreditation of Laboratory Animal Care International. The Director of Yerkes reports to the Executive Vice President for Health Affairs of Emory University.

The next Director will be a distinguished scientist with a strong record of achievements in a nonhuman primate-based field of research and will have documented skills in administration and program development, as well as an aptitude for fostering community engagement with integrity and mutual respect. It is expected that the new Director will arrive well before the departure of the current Director, Dr. Stuart Zola, in order to ensure continuity and a smooth transition as the organization approaches the renewal date for its NIH base-grant application in the fall of 2015.

Korn/Ferry International is assisting Robert W. Woodruff Health Sciences Center with this important search. Please forward, as soon as possible, nominations of appropriate candidates to: **Warren E. Ross, M.D., c/o Betsy Messina** (betsy.messina@kornferry.com), Korn/Ferry International, 1835 Market Street, Suite 2000, Philadelphia, PA 19103.

Robert W. Woodruff Health Sciences Center is an Equal Opportunity/Affirmative Action Employer



KORN/FERRY INTERNATIONAL



UNC
LINEBERGER

The UNC Lineberger Comprehensive Cancer Center, in collaboration with departments in the UNC Chapel Hill School of Medicine, seeks outstanding candidates for multiple faculty positions at all levels and at all ranks to expand our tumor immunology / immunotherapy program. These positions coincide with development of a GMP facility for delivering novel immunebased approaches to treat patients with cancer. This broadbased recruitment seeks candidates in all areas of tumor immunology research; however, applicants should have a track record of clinical/translational or basic research focused on enhancing the adaptive and/or innate immune response for the treatment of specific malignant diseases. Applicants should also have a strong record of recent accomplishments as a postdoctoral fellow or sustained productivity as an established faculty member. Appointment and rank in an academic department will be determined by the applicant's qualifications. Faculty are expected to compete for extramural funding from the National Institutes of Health and other agencies. Applications will be reviewed beginning **December 1, 2013** and until the positions are filled.

Educational Requirements: Doctoral Degree

Qualifications and Experience: Doctoral Degree

Apply online at <http://unc.peopleadmin.com/postings/32265>. Please provide a CV, a research statement, and a list of four references.

The University of North Carolina at Chapel Hill is an Equal Opportunity Employer. Women and minorities are strongly encouraged to apply and self-identify on their application.

Executive Director

Singapore Immunology Network (SIgN), Singapore

The Agency for Science, Technology and Research (A*STAR) is a diverse scientific community comprising more than 4,000 research scientists and engineers from over 60 countries world-wide. A*STAR seek a visionary leader to head the Singapore Immunology Network (SIgN), one of 20 research entities which together foster world-class scientific research and talent and contribute to a vibrant international knowledge-based economy. This is an outstanding opportunity to forge a multi-disciplinary and multi-cultural research environment in a nation dedicated to developing commercially viable and successful scientific R&D in collaboration with global academic and industrial partners.

SIgN was established to advance human immunology research and participate in international efforts to combat major health problems. Funded primarily by the A*STAR Biomedical Research Council (BMRC), research programmes at SIgN currently focus on six areas:

- **Infectious diseases**
- **Cancer immunology**
- **Inflammatory, allergic and auto-immune diseases**
- **Immunoregulation**
- **Clinical immunomonitoring**
- **Therapeutic human antibodies**

As Executive Director of SIgN, you will be responsible for driving and developing these programmes, maintaining world class research quality, maximising their translational impact and helping to shape Singapore's biomedical R&D landscape.

Core responsibilities will include providing scientific leadership to researchers and support staff and orchestrating the Institute's operations through scientific programme development and resource management. You will oversee SIgN's engagements and collaborations with the wider research and clinical communities in Singapore and beyond and play a key role in aligning SIgN's activities and resources to A*STAR's mission of fostering world-class scientific research and talent.

You should be an outstanding, visionary scientist and proven leader, prepared to commit to nurturing and developing the research talents of others, bringing benefit to Singapore medicine and its economy. A track record of scientific leadership in a research-based organisation of scale is essential. Your chosen field of biomedical science need not be restricted to human immunology: A*STAR would welcome interest from specialists from related, applied fields of biomedical science, who are similarly equipped with a broad understanding of the biology of human diseases. Familiarity with industry R&D needs and partnering in order to advance translational research towards effective health outcomes would be a distinct advantage.

More information on A*STAR and SIgN can be found at www.a-star.edu.sg and www.sign.a-star.edu.sg respectively.

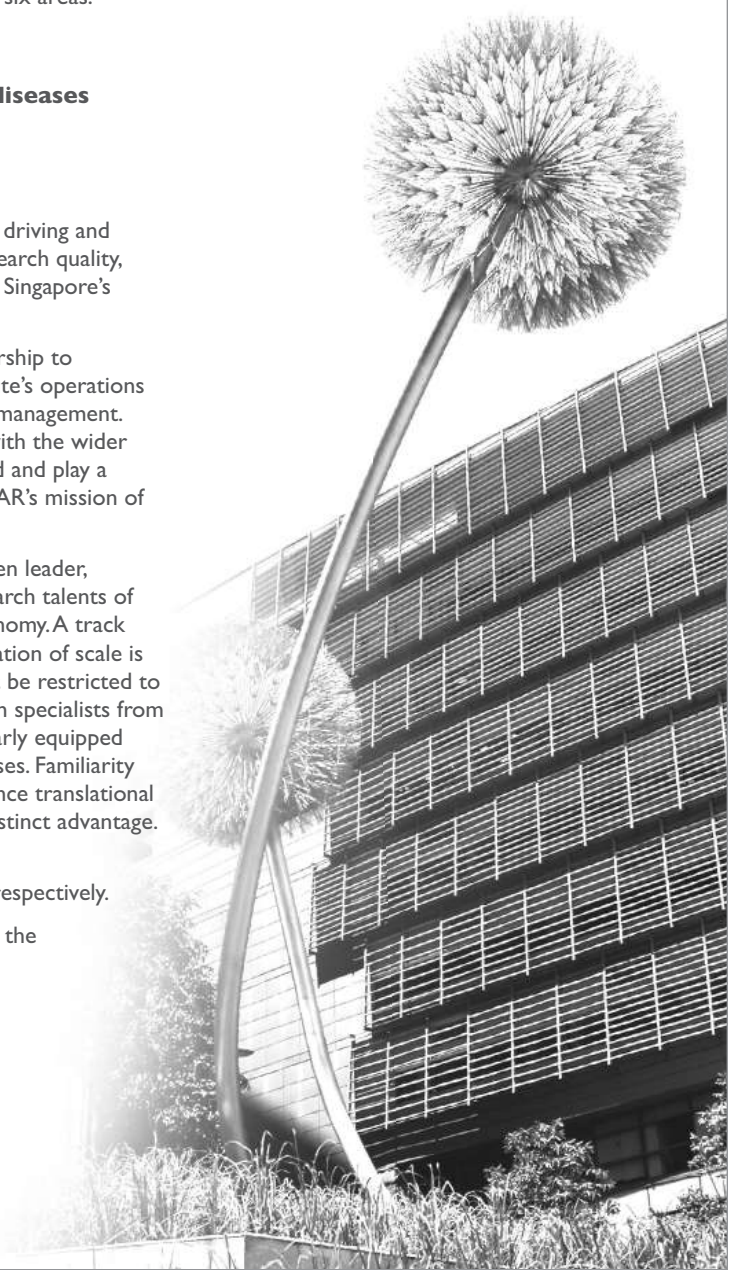
To further your interest, please contact Dr Kevin Young of the RSA Group at kevin.young@thersagroup.com

The closing date for applications is November 15th 2013.

RSRA



SIgN





Tufts
UNIVERSITY

School of Medicine

Faculty Positions in Virology

The Department of Molecular Biology and Microbiology at Tufts University School of Medicine, Boston MA (<http://medicine.tufts.edu/Education/Academic-Departments/Basic-Science-Departments/Molecular-Biology-and-Microbiology>) is undergoing a major expansion and will be adding tenure-track faculty positions. Positions are available at the **Assistant, Associate, and Full Professor** levels. The successful candidates will build on the core strengths of the Department and focus on the study of viruses, including, but not limited to, structural virology, virus-cell interaction, viral pathogenesis, host-virus evolution, and global health and emerging viruses. We are seeking candidates who use innovative approaches to investigate problems at molecular, cellular and organismal levels. We offer generous start-up packages, modern laboratory space and a highly collaborative environment with opportunities for both basic and translational research. A joint appointment at Tufts Medical Center is possible for individuals with clinical training.

Applicants should hold a Ph.D. and/or M.D. degree, and have an excellent track record of research productivity. Successful candidates will be expected to establish and direct a thriving, high-quality independent research program and to contribute to graduate and medical education. Please submit electronic applications including a CV, a statement of research interests and the names and contact information for at least three references to <https://academicjobsonline.org/ajo/jobs/2973>. Review of applications will begin **November 15, 2013** and continue on a rolling basis thru March 31, 2014. Inquiries, but not application materials, may be directed to MBMFacultyRecruitment@tufts.edu.

Tufts University is an Equal Opportunity Affirmative Action Employer. We are committed to increasing the diversity of our faculty. Applications from women and members of underserved minority groups are encouraged.



HARVARD
School of Engineering
and Applied Sciences

Faculty Positions in Environmental Science and Engineering

The Harvard School of Engineering and Applied Sciences (SEAS) seeks applicants for positions at the tenure-track or tenured level in the field of Environmental Science and Engineering.

The School of Engineering and Applied Sciences (SEAS) at Harvard University is embarking on a broad initiative within the area of Environmental Science and Engineering. Our goal is to expand and strengthen undergraduate and graduate education and research on problems that affect the environment, on scales from local to global and spanning basic and applied sciences and engineering. Highlighted topics include but are not limited to: environmental and energy science, technology, air and water quality, hydrology, natural hazard mitigation, atmospheric science, oceanography, glaciology, and climate science. A key aspect of our strategy in the development of teaching and research initiatives at Harvard has been the close relationship between SEAS and the other science departments at the University such as physics, chemistry and chemical biology, earth and planetary sciences, organismic and evolutionary biology, etc.

Candidates are required to have a doctorate or equivalent terminal degree by their start date. In addition, we are seeking candidates of exceptional scientific and/or engineering talent who have demonstrated excellence in teaching and experimental, observational, theoretical, or computational research in one or more of these areas and who would be eager to participate in an expanding ESE undergraduate curriculum.

Required application documents include a cover letter, curriculum vitae, copies of up to three representative papers, a research statement, a teaching statement, and names and contact information for at least three references. We encourage applicants to apply by **December 15, 2013**, but applications will be accepted until the positions are filled. Applicants will apply on-line at <http://academicpositions.harvard.edu/postings/5011>

Harvard University is an Equal Opportunity/Affirmative Action Employer. Applications from women and underrepresented minority candidates are strongly encouraged.



UNIVERSITY OF
LIVERPOOL

Faculty of Health and Life Sciences Institute of Translational Medicine Department of Molecular and Clinical Cellular Physiology Chair of Preclinical Imaging Salary Negotiable

The University of Liverpool is investing in state-of-the-art imaging platforms, including high-field MRI and photoacoustic imaging, as part of an initiative to establish a Centre for Preclinical Imaging (CPI). An important aim of the CPI will be to support the research of the newly-established UK Regenerative Medicine Platform Safety Hub, which is led by Liverpool (www.ukrmp.org.uk). We are seeking a suitable individual at Professor level to help develop, direct and enhance this exciting facility. You must be able to demonstrate the drive, expertise, experience and vision to create a world-class imaging resource serving both the local and national research communities. You will combine internationally-recognised research excellence with the management and leadership skills required to establish and maintain collaborative research programmes.

Job Ref: A-579289/S

Closing Date: 1 November 2013

For full details, or to request an application pack, visit www.liv.ac.uk/working/job_vacancies/ or e-mail jobs@liv.ac.uk Please quote job ref in all enquiries.

COMMITTED TO DIVERSITY AND
EQUALITY OF OPPORTUNITY



Stonewall
DIVERSITY CHAMPION



The University of New Mexico

Quantitative Ecologist The University of New Mexico Department of Biology

The Department of Biology at the University of New Mexico invites applications for a full-time, tenure-track probationary appointment in quantitative ecology at the **Assistant Professor** level, beginning in Fall 2014.

Minimum Qualifications include a Ph.D. and post-doctoral experience in ecology or a related discipline by the start of the position. For complete details or to apply, please visit: <https://unmjobs.unm.edu/> and reference the **posting number 0822368**. Best consideration date is **November 1, 2013**. Review of applications will commence on **November 2, 2013**. The position will remain open until filled.

Preferred Qualifications: We are particularly interested in ecologists who use quantitative methods to develop ecological theory and address research questions at larger spatial or temporal scales in terrestrial or aquatic systems. The successful candidate will demonstrate excellence in research as evidenced by pre- and post-doctoral work; have a strong publication record in peer-reviewed journals; be committed to establishing an internationally recognized and externally funded research program in the general areas of theoretical, quantitative, or spatial ecology (e.g. Landscape Ecology, Ecological Modeling, Macroecology). Preference is given to candidates whose research and teaching areas complement existing strengths in the Department of Biology. The successful candidate will demonstrate a commitment to excellence in teaching at the undergraduate through graduate level in a minority majority institution and show enthusiasm for working in a broadly collaborative biology department with diverse research strengths.

To apply, applicants must submit a letter of interest, curriculum vitae, three recent publications, statements of research and teaching interests, and a list of names and contact information of five referees. All materials must be submitted directly to <https://unmjobs.unm.edu/> by the best consideration date. Questions can be directed to **Dr. Scott Collins** (scollins@sevilleta.unm.edu).

The University of New Mexico is an Equal Opportunity/Affirmative Action Employer and Educator. Women and underrepresented minorities are encouraged to apply.



Penn Nano Clusters Hiring

The School of Engineering and Applied Science (SEAS) at the University of Pennsylvania seeks to build interdisciplinary faculty clusters of eminence at the forefront of nanotechnology. The newly opened Krishna P. Singh Center for Nanotechnology is a \$100M facility integrating state-of-the-art nanocharacterization and nanofabrication facilities. This second phase seeks numerous hires who will comprehensively span forefront measurement, novel phenomena, innovative devices, and integrated systems. Successful candidates will be expected to couple with existing resources to synergistically build new areas of international impact.



Broad themes of interest include:

- Emerging fields at the interface of nano and biotechnology,
- Advanced nanopores pushing the frontiers of multi modal interactions, in-situ control and/or ultra fast dynamics;
- Coupled phenomena providing new paradigms, particularly under extreme environments including ultrafast time scales, ultra-small dimensions and intense energy fluxes;
- Design and fabrication of multifunctional devices that exploit such coupled phenomena for novel applications;
- Integrated systems for application in nano-enabled computation, energy technologies and nanomanufacturing.

Candidates will be expected to robustly utilize and further contribute to the development of experimental capabilities in the Singh Center, as well as to acquire and develop their own innovative experimental platforms. Read more about the Singh Center at <http://www.nano.upenn.edu/>. Candidates will also be expected to advance our creative educational programs at both the undergraduate and graduate level. Applicants with industrial experience or collaborations, and track records that include successful translational research programs and technology transfer are particularly encouraged and should highlight these accomplishments in their application.

Appointments in this second round of hiring will be at the Associate or Full Professor level and applicants must have research and educational track records to merit an appointment with tenure. Successful candidates will be invited to participate in recruiting future faculty at all levels to further contribute to this long-term cluster hiring initiative, which builds on

Penn's exemplary record in interdisciplinary research that integrates knowledge at the forefront of discovery.

Applicants should submit their applications electronically at <http://www.nano.upenn.edu/about/hiring-initiative/>

Information about the School of Engineering and Applied Sciences is available at <http://www.seas.upenn.edu/>.

The University of Pennsylvania values diversity and seeks talented students, faculty and staff from diverse backgrounds and does not discriminate on the basis of race, color, sex, sexual orientation, gender identity, religion, creed, national or ethnic origin, citizenship status, age, disability, veteran status or any other legally protected class status in its employment practices.



Group Leaders in Cell Biology and in Cellular Imaging

The Cell Biology Division of the MRC Laboratory of Molecular Biology (LMB) invites applications for two tenure-track Group Leader posts. The LMB has an exceptional record of ground-breaking research, and has recently moved into a new building with outstanding facilities including those for light and electron microscopy, as well as electronic and instrumentation workshops. The two posts are:

(1) Research Group Leader in Cell Biology Reference IRC111673

To lead a new research group in the Cell Biology Division. We seek someone addressing fundamental questions in any area of cell biology. Cellular damage control and aging are areas of particular interest, but the primary selection criteria are scientific excellence and potential for synergy with our existing research programmes.

(2) Research Group Leader in Cellular Imaging Reference IRC111773

To lead a new imaging-based research group in the Cell Biology Division. We seek someone developing or applying advanced light or electron microscopy techniques that are relevant to studying fundamental biological problems at a cellular level. We are particularly interested in candidates with potential for synergy with our existing research programmes.

Applicants should hold a PhD and have an excellent publication record with outstanding potential for independent research. Substantial core funding sufficient to run a small research group will be provided.

Find out more about the LMB's Cell Biology Division at:

www2.mrc-lmb.cam.ac.uk/research/cb/

Informal enquiries can be made to Sean Munro, email: sean@mrc-lmb.cam.ac.uk

The salary for these posts is £40,540 - £48,000 per annum and will be supported by 30 days annual leave entitlement, an optional MRC final salary pension scheme and excellent on-site sports and social facilities.

Applicants should visit <http://www.topcareer.jobs/> using the relevant reference number detailed above for further details on these roles and how to apply. Submissions should include a covering letter and full CV, an outline of current research interests (1 page) and a proposal for future research (up to 2 pages), along with the names and addresses of three referees who have agreed to be contacted prior to interview.

The posts will remain open until they are filled but applications received by 4th November 2013 will receive priority.

For further information about the MRC visit: www.mrc.ac.uk

The MRC is an Equal Opportunities Employer

No agencies please

UC DAVIS

The University of California at Davis is pleased to announce recruitment for a tenure-track faculty position in Quantitative Genetics. The successful candidate will join the Department of Animal Science in the College of Agricultural and Environmental Sciences at the rank of Assistant Professor. Criteria for appointment include: a Ph.D. or equivalent, a strong interest in Quantitative Genetics of domestic animals relevant to animal agriculture, a record of excellence in scholarly research, and demonstrable potential to establish a competitively-funded research program relevant to animal genetics and animal breeding. The appointee will be responsible for teaching undergraduate courses including quantitative and population genetics of domestic animals, be actively involved in undergraduate advising, curricular development and department and university service. The appointee is also expected to guide and mentor graduate students and participate in research and outreach programs consistent with the mission of the CA Agricultural Experiment Station.

Applicants should submit materials via the following website:
<https://recruit.ucdavis.edu/apply/JPF00149/>

Additional inquiries can be directed to **Professor J.F. Medrano, Recruitment Advisory Committee Chair, Department of Animal Science, One Shields Avenue, University of California, Davis, CA 95616, telephone (530) 752-6786, jfmedrano@ucdavis.edu**. The position will remain open until filled but to ensure consideration, applications should be received by **December 1, 2013**.

UC Davis is an Affirmative Action/Equal Employment Opportunity Employer and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, veterans, and individuals with disabilities.

UNIVERSITY of HOUSTON

COLLEGE OF PHARMACY

Faculty Position in Immunology Department of Pharmacological and Pharmaceutical Sciences

The Department of Pharmacological and Pharmaceutical Sciences at the University of Houston is accepting applications for a tenure-track faculty position in Immunology at either the Associate Professor or Professor level. The University of Houston is Carnegie-designated Tier1 public research university and also is a member institution of the world-renowned Texas Medical Center which houses major academic research programs in cancer, genetics, and neuroscience with a major focus on translational research. The successful candidate will be provided lab space will receive a start-up package commensurate with their qualifications. Department faculty members teach in the Pharm.D. professional program and also participate in departmental Ph.D. programs in Pharmacology and Pharmaceuticals. The search is focused on adding expertise in the immunology, immunotherapy and vaccines along with other areas relevant to drug development.

Eligible candidates must have an earned doctoral degree, postdoctoral experience and a strong track record for establishing and maintaining an excellent extramurally-funded research program. Applications will be accepted until the position is filled, although an early application is recommended. Interested individuals should send a letter describing his/her research program, a curriculum vitae and the names of three references with postal and Email addresses, telephone and FAX numbers electronically in PDF format to:

Tahir Hussain, Ph.D., Chair, Search Committee
c/o ppsimmun@uh.edu
University of Houston
College of Pharmacy
Houston, TX, 77204-5037

Web site: <http://www.uh.edu/pps/ppsm/index.html>

The University of Houston is an Affirmative Action/Equal Opportunity Employer. Minorities, women, veterans, and person with disabilities are encouraged to apply.



Harvard University Faculty of Arts and Sciences Department of Organismic and Evolutionary Biology

TENURE-TRACK PROFESSOR

Position description: The Department of Organismic and Evolutionary Biology seeks to appoint a tenure-track professor in the field of invertebrate biology and/or invertebrate paleontology, whose research includes any clade(s) of living or extinct invertebrates and emphasizes one or more of the following areas: evolution, systematics, ecology, physiology, development, genetics, genomics or environmental biology. We seek an outstanding scientist who will establish an innovative research program, teach both undergraduate and graduate students, and interface with relevant programs throughout Harvard. The successful candidate will be appointed to one of two vacant curatorial positions in the Museum of Comparative Zoology (MCZ) and share oversight responsibilities for the museum's invertebrate collections. Additional faculty appointments in invertebrate biology and invertebrate paleontology are anticipated in future years to sustain Harvard's strength in these areas.

Basic qualifications: Doctorate required by expected start date.

Additional qualifications: Demonstrated excellence in teaching and research is desired, as is postdoctoral experience.

Special instructions: Please submit the following materials through the ARIeS portal (<http://academicpositions.harvard.edu/postings/5045>), no later than November 11th, 2013: 1. Cover letter; 2. *Curriculum vitae*; 3. Teaching statement; 4. Research statement; 5. Names and contact information of 3-5 references; 6. 3-5 representative publications

Contact information: Further information about OEB and MCZ are available at <http://www.oeb.harvard.edu> and <http://www.mcz.harvard.edu>. Harvard University, Organismic and Evolutionary Biology, Chair, Search Committee, 26 Oxford Street, Cambridge, MA 02138; Contact email: fac-search@oeb.harvard.edu.

Harvard is an Equal Opportunity/Affirmative Action employer. Applications from women and minorities are strongly encouraged.



Harvard University Faculty of Arts and Sciences Department of Organismic and Evolutionary Biology

TENURE-TRACK PROFESSOR

Position description: The Department of Organismic and Evolutionary Biology (OEB) and the Harvard University Herbaria (HUH) seek to appoint a tenure-track faculty member in the Department of OEB (<http://www.oeb.harvard.edu/>) in the field of plant diversity and evolution. We seek an outstanding scientist who will teach both undergraduate and graduate students, and who is engaged in an innovative research program. The successful candidate will also be appointed as curator in the HUH and share responsibilities for the HUH's physical and digital collections. We are especially interested in individuals who undertake field and laboratory research in plant phylogenomics, genetics, development, speciation, and/or biogeography.

The Harvard University Herbaria (HUH; <http://www.huh.harvard.edu>) house one of the largest and most comprehensive collections of dried plant and fungal specimens in the world. These specimens are the key to our knowledge of plants and serve as a permanent reference to the diversity of life on earth. During the last five years, the HUH has embarked on an ambitious plan to enhance its scientific mission through a series of key improvements in its laboratories, bioinformatics and collections infrastructure, and environmental controls. This tenure-track position is part of this broader initiative.

Basic qualifications: Doctorate required by expected start date.

Additional qualifications: Demonstrated excellence in teaching and research is desired, as is postdoctoral experience.

Special instructions: Please submit the following materials through the ARIeS portal (<http://academicpositions.harvard.edu/postings/5008>), no later than November 15th, 2013: 1. Cover letter; 2. *Curriculum vitae*; 3. Teaching statement; 4. Research statement; 5. Names and contact information of 3-5 references; 6. 3-5 representative publications. Contact information: Further information about OEB and HUH is available at <http://www.oeb.harvard.edu> and <http://www.huh.harvard.edu>. Contact email: fac-search@oeb.harvard.edu.

Harvard is an Equal Opportunity/Affirmative Action Employer. Applications from women and minorities are strongly encouraged.

Joint Faculty Appointments for a New Initiative

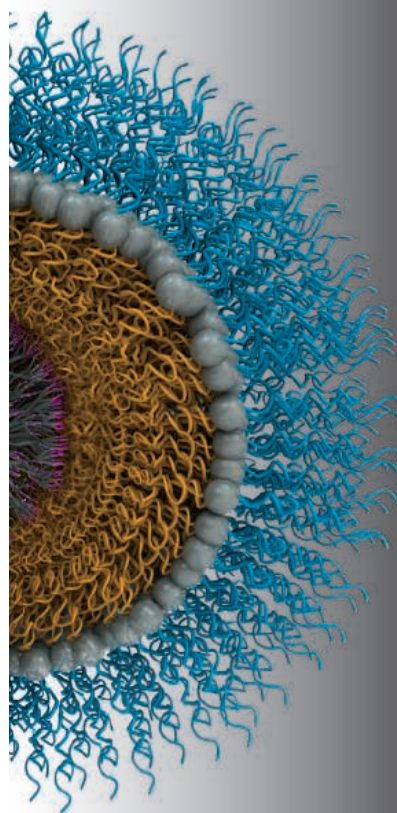
The Institute for Molecular Engineering and the Institute for Genomics and Systems Biology at the University of Chicago invite applications for faculty positions at all ranks. Areas of interest include, but are not limited to:

- Synthetic and systems biology
- Multiscale modeling and prediction
- Bioengineering
- Bioinformatics
- Imaging, genomics
- Regenerative medicine
- Medical diagnostics and therapeutics

While appointment at any level is possible, we particularly invite applications for tenure-track positions at the Assistant and Associate Professor ranks. The appointment will be at the Institute for Molecular Engineering with membership at the Institute for Genomics and Systems Biology. Candidates must have a doctoral degree in a relevant field of study and have an outstanding research record. Successful candidates will be expected to establish and maintain a strong research program and to teach at the graduate level.

To apply, please visit the University of Chicago's Academic Career Opportunities website (academiccareers.uchicago.edu), posting number 01887. The review of the applications will start on Dec. 1, 2013 and continue until the position is filled.

The University of Chicago is an Affirmative Action/Equal Opportunity Employer.



Faculty Positions

INSTITUTE FOR ADVANCED COMPUTATIONAL SCIENCE

Stony Brook University is one of America's most dynamic public universities, a center of academic excellence and a leader in health education, patient care and research. Listed among the top 1 percent of all universities in the world by the *Times Higher Education World University Rankings*, Stony Brook is home to more than 24,100 undergraduate, graduate and doctoral students and more than 14,000 faculty and staff, including those employed at Stony Brook Medicine, Long Island's premier academic medical center and teaching hospital. With 603 beds, Stony Brook University Hospital is the region's only tertiary care center and Regional Trauma Center. The University is a member of the prestigious Association of American Universities and co-manager of nearby Brookhaven National Laboratory.

Applications are invited for four tenure-track faculty positions in applied mathematics and computer science in the Institute for Advanced Computational Science (IACS) at Stony Brook University – two at the assistant professor level and two at the full professor level with named chairs and significant individual endowments within the institute. Candidates wishing to apply should have a doctoral degree in Computer Science or Applied Mathematics, though a degree in related fields may be considered. Ten years of faculty or professional experience is required for the two senior positions along with a demonstrated record of publications and research funding. A demonstrated record of publications and a demonstrated potential for research funding is required for the junior faculty position. The selected candidate is expected to participate in interdisciplinary program development within the Institute and to establish a research program with a solid funding base through both internal and external collaborations. Of specific interest is research in, for example, programming models, algorithms or numerical representations that advance scientific productivity or broaden the benefit and impact of high-performance computing. The selected candidates will have access to world-class facilities including those at nearby Brookhaven National Laboratory.

The Institute for Advanced Computational Science (iacs.stonybrook.edu) was established in 2012 with an endowment of \$20 Million, including \$10 Million from the Simons Foundation. The current eight faculty members will double in number over the next few years to span all aspects of computation with the intent of creating a vibrant multi-disciplinary program. IACS seeks to make sustained advances in the fundamental techniques of computation and in high-impact applications including engineering and the physical, life and social sciences. Our integrated, multidisciplinary team of faculty, students and staff overcome the limitations at the very core of how we compute, collectively take on challenges of otherwise overwhelming complexity and scale, and individually and jointly define new frontiers and opportunities for discovery through computation. In coordination with the Center for Scientific Computing at Brookhaven National Laboratory, our dynamic and diverse institute serves as an ideal training and proving ground for new generations of students and researchers, and provides computational leadership and resources across the SBU campus and state of New York.

The search will remain open until suitable candidates are found. Optimal start date is Fall 2014. All candidates must submit the required documentation online through the link provided below. Please input a cover letter, copy of your curriculum vitae, research plan (max. two pages), publication list, funding history, one-page statement of your teaching philosophy and three reference letters to <https://academicjobsonline.org/ajo/jobs/3072>.

Alternatively, applicants may submit a cover letter, résumé/CV, research plan (max. two pages), publication list, funding history, one-page statement of teaching philosophy and three reference letters to:

Search Chair, Institute for Advanced Computational Science, Heavy Engineering, Suite 230
Stony Brook University, Stony Brook, NY 11794-5250

Stony Brook University/SUNY is an affirmative action, equal opportunity educator and employer.



Stony Brook
University



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/sciencecareers



Alfred Kinsey

Changed the world's understanding of human sexuality in his century.
Seeking a new leader for our century.

Indiana University seeks the next director of the world-renowned **Kinsey Institute for Research in Sex, Gender, and Reproduction**. Following in the steps of Dr. Alfred C. Kinsey and his successors, the next leader will help advance rigorous, high-quality research and global education for sexual health as a member of the IU Bloomington faculty.

Established in 1947, the Kinsey Institute has been a trusted source for investigating and informing the world about critical issues in sex, gender, and reproduction for more than 60 years. The next leader may come from a variety of academic or professional backgrounds.

For more information, visit:

www.kinseyinstitute.org/search

Priority application deadline:

December 2, 2013

Nomination submissions:

kinsey-search@indiana.edu



PURDUE UNIVERSITY Faculty Position in Evolutionary Ecology Department of Biological Sciences

The Department of Biological Sciences, Purdue University, invites applicants for a tenure-track faculty position in the area of Evolutionary Ecology. We seek candidates whose research integrates the fields of ecology and evolutionary biology through investigations in subfields such as community and conservation ecology, population and evolutionary genetics, and evolutionary dynamics. Applicants must have a Ph.D. or equivalent in ecology or evolutionary biology; postdoctoral experience preferred. We expect to fill one academic-year appointment at the Assistant Professor level. The successful applicant is expected to conduct research to address fundamental questions in an area listed above, teach undergraduate and graduate students, and contribute to activities in the Department of Biological Sciences, Department of Forestry and Natural Resources, and interdisciplinary programs.

The Department of Biological Sciences has over 50 faculty members conducting research in a wide range of fields including evolutionary biology, ecology, behavior, microbiology/virology, structural biology, developmental biology, molecular/cell biology, and bioinformatics. Further information about the Department is available at <http://www.bio.purdue.edu/>. The successful candidate will have opportunities to interact with allied scientists across the University, including colleagues in Purdue's Center for the Environment, Climate Change Research Center, and Bindley Bioscience Center. Purdue has first-rate laboratory and computational facilities for analytical and systems work, and a wide variety of field facilities in the surrounding landscape including the Ross Biological Reserve.

Applications must be submitted electronically to <https://hiring.science.purdue.edu> as a PDF file that includes a detailed curriculum vitae, names and addresses of three referees, a 2-3 page summary of research interests, and a one-page teaching statement. Inquiries should be directed to **Evolutionary Ecology Search Committee, Department of Biological Sciences, Purdue University, 915 W. State St., West Lafayette, IN 47907-2054**. Review of applications will begin **November 8, 2013** and continue until the position is filled. A background check will be required for employment in this position.

Purdue University in an Equal Opportunity/Equal Access/Affirmative Action Employer fully committed to achieving a diverse workforce.



Florida State University

Faculty Recruitment for a Multidisciplinary Initiative in Coastal and Marine Research

The President and the Provost of Florida State University are pleased to announce a major initiative to develop a multidisciplinary group studying the short- and long-term dynamics of coastal ecosystems, especially with respect to the interconnectivity among biotic and abiotic components of terrestrial and marine environments. This strategic effort seeks to recruit as many as nine tenure-track/tenured faculty, open with respect to rank. Faculty will have academic appointments in either the Department of Earth, Ocean and Atmospheric Sciences (EOAS) or the Department of Biological Science. Some of the new faculty hires can be based at the FSU Coastal and Marine Laboratory (FSUCML).

Successful candidates are expected to interact synergistically with research programs in departments and interdisciplinary programs across the University, as well as develop new areas of interactions through research and graduate and undergraduate teaching. The sustained pursuit and growth of collaborative, externally-funded research programs are explicit goals of this initiative.

This is a very broad search and we encourage applications from candidates trained in the physical and life sciences who work on subjects related to coastal and marine systems. Areas of interest include, but are not limited to, ecosystem or community ecology, conservation biology, invertebrate/benthic ecology, plant or algal ecology, fisheries biology, marine mammalogy, (bio)geochemistry, geology, climatology, hydrology, shelf circulation processes and biotic/abiotic system modeling.

Successful candidates will be offered highly competitive salaries and start-up packages as well as access to state-of-the-art research space, instrumentation, high performance computing and other facilities. Faculty are expected to integrate into existing coastal and marine ecosystem research in EOAS, Biological Science and the FSUCML, with the potential to interact with the Center for Ocean-Atmospheric Prediction Studies and the Geophysical Fluid Dynamics Institute. Further resources and support are available through existing programs with other institutions, including the Florida Climate Institute, the Deep-C Consortium and the National Estuarine Research Reserve System.

Applicants should provide a letter of application, full curriculum vitae, the names and contact information of three professional references and a two-page narrative describing their research interests that should include a statement as to how the candidate would complement this multidisciplinary effort at Florida State University. Application documents must be combined into a single PDF file and sent electronically to ecosystems.search@fsu.edu. Review of applications and nominations will begin **November 15, 2013**. Additional information about the programs at FSU and this faculty search can be obtained at http://www.research.fsu.edu/ecosystems_search/.

Florida State University is committed to the diversity of its faculty, staff, and students, and to sustaining a work and learning environment that is inclusive. Women, minorities, and people with disabilities are encouraged to apply. FSU is an Equal Opportunity/Access/Affirmative Action Employer.



Join CIFOR and make a difference

The leading global institute on science for forests and people is seeking a visionary and dynamic

Director of Governance Research (Ref. 1333)

The Center for International Forestry Research (CIFOR) advances human well-being, environmental conservation, sustainable development and equity by conducting research to inform and support policies and practices that affect people, forests and landscapes. CIFOR is headquartered in Indonesia, with offices across Asia, Africa and Latin America, and is a member of the CGIAR Consortium.

The new Director will be responsible for the development, management, delivery and scientific quality of CIFOR's engagements in governance research. This includes institutional arrangements, public and private finance, gender, legal frameworks, law enforcement, land and forest tenure reforms, collaborative forest and landscape management, and climate change — all of which will be addressed from a cross-sectoral and broad development perspective.

The Director will ensure coordination with CIFOR's portfolios on livelihoods and environmental research as well as with relevant CGIAR Research Programs. S/he will manage a team at HQ and supervise the scientific work of researchers globally. As a member of CIFOR's senior management, the Director will also fulfill center-wide roles, such as managing strategic partnerships, convening conferences and representing CIFOR at key events.

The successful candidate will be an internationally recognized scientist in her or his field, will have strong leadership skills, and will share our mission and commitment to professionalism, innovation, impact and collaboration. The Director will have a PhD in a relevant discipline and have extensive experience in research and managing research in an international, multicultural and multidisciplinary environment.

CIFOR is an equal opportunity employer with a strong belief that staff diversity contributes to excellence. Women are strongly encouraged to apply. To learn more about us, this position and how to apply, visit www.cifor.org.

**CIFOR is an equal opportunity employer.
Staff diversity contributes to excellence.**

POSITIONS OPEN

ECOLOGICAL OR EVOLUTIONARY THEORY

The Florida State University (FSU) Department of Biological Science invites applications for a tenure-track faculty position, at the **ASSISTANT PROFESSOR** level. We seek a creative and interactive individual using theory to answer basic questions in ecology, evolution, or the interface of these fields, and whose research will enhance existing strengths in our Ecology and Evolution research group. Candidates should demonstrate high potential for collaborations with empiricists, externally funded research, and engaging instruction at both graduate and undergraduate levels. More information about the E&E group and department can be found at **website: <http://www.bio.fsu.edu/ee/>**.

Please submit an electronic application as a single PDF document containing a cover letter, curriculum vitae, and statements of research and teaching interests to **e-mail: jobs@fsu.edu**, Job ID 36276, and request three letters of reference to be sent to the same address. Applications must be received by November 1, 2013. FSU is an Equal Opportunity/Access/Affirmative Action Employer committed to enhancing the diversity of its faculty and students. Individuals from traditionally underrepresented groups are encouraged to apply.

ASSISTANT PROFESSOR OF BIOLOGY Immunology/Microbiology

The Biology Department at Missouri State University anticipates an August 2014 opening for a tenure-track Assistant Professor in Immunology/Microbiology. Requirements include a Ph.D. in Microbiology or related area, peer-reviewed publications in immunology, and excellent communication skills. Duties include teaching courses in microbiology, immunology, and specialty; graduate and undergraduate advisement; and externally funded research. A letter of application (including a commitment to working with diverse student populations), curriculum vitae, names, and contact information for three references, a statement of teaching experience and interests, and copies of all university transcripts should be submitted online at **website: <https://jobs.missouristate.edu>**. *Employment will require a criminal background check at University Expense.* Application review begins on 7 November 2013 and continues until position is filled. Starting date is 11 August 2014. Direct queries to **e-mail: pauldurham@missouristate.edu**. Equal Opportunity/Affirmative Action.

Help employers
find you. Post
your resume/cv.

Science Careers

From the journal *Science*



www.ScienceCareers.org

Get your questions answered.

Careers Forum

www.ScienceCareers.org

**Download
your free copy
today.**

ScienceCareers.org/booklets



From technology specialists to patent attorneys to policy advisers, learn more about the types of careers that scientists can pursue and the skills needed in order to succeed in nonresearch careers.

Science Careers

From the journal *Science*





澳門大學
UNIVERSIDADE DE MACAU
UNIVERSITY OF MACAU

International Search for Chair Professors and Distinguished Professors

The University of Macau is a leading higher educational institution in Macao and is making strides towards becoming internationally recognized for its excellence in teaching, research and service to the community. The University is growing rapidly with a number of new strategic initiatives including the relocation to a new campus and the establishment of the largest Residential College system in Asia. The new campus is 20 times larger than the present one with a projected fast growth of student intake and faculty size. English is the University's working language.

We plan to develop a strong team of top-notch scholars to help us realize our vision. Applications are therefore invited from those with excellent academic achievements in the following disciplines:

- * Business and Management * Education * Law
- * Liberal Arts and Humanities * Social Sciences
- * Mathematics, Sciences and Engineering
- * Health Sciences * Chinese Medicines

Remuneration and appointment rank offered will be competitive and commensurate with the successful applicants' academic qualification, current position and professional experience. The current local maximum income tax rate is 12% but is effectively around 5% - 7% after various discretionary exemptions.

For details about the above open positions and related information, please visit the following websites:

Job vacancy website: <http://www.umac.mo/vacancy>

University website: <http://www.umac.mo>

Macao government website: <http://www.gov.mo>

Applicants please state the specific disciplines applied for and quote the reference no.: ACAD/PROF/09/2014 in their application and send it by email to the below address preferably on or before 20 October 2013. Review of applications will commence immediately and continue until the positions are filled. Applicants may consider their applications not successful if they were not invited for an interview within 3 months of application.

Human Resources Office

University of Macau, Av. Padre Tomás Pereira, Taipa, Macau

Website: <https://isw.umac.mo/recruitment>;

Email: senior.academic@umac.mo

Tel: +853 8397 8593 or +853 8397 8592;

Fax: +853 8397 8694

The effective position and salary index are subject to the Personnel Statute of the University of Macau in force.

The University of Macau reserves the right not to appoint a candidate. Applicants with less qualification and experience can be offered lower positions under special circumstances.

Personal data provided by applicants will be kept confidential and used for recruitment purpose only

University of Macau -
An ideal place to pursue your career

<http://www.umac.mo>



PRINCETON
Neuroscience
INSTITUTE

Princeton University is committed to the continued expansion and development of neuroscience on its campus. In addition to other outstanding resources, a newly constructed 248,000 square foot building will open in late fall 2013 housing state-of-the-art facilities for the full range of neuroscientific methods, including human brain imaging, cellular and circuit level imaging in model organisms, structural neurobiology, studies of non-human primates, and computation and theory. These new facilities will support the continued growth of neuroscience at Princeton, including the three new positions described below. We are seeking qualified individuals working at all levels of neuroscience, to study development, learning, memory, perception, attention and higher level cognitive functions in humans and non-human species.

Tenured Full Professor Position

The Princeton Neuroscience Institute invites applications for a tenured appointment at the associate or full professor level to begin on or after September 2014.

Key selection criteria will be research excellence, originality of science, future impact on the field of neuroscience and related disciplines, and leadership capabilities. Applicants must have an excellent record of research productivity and demonstrate the ability to develop a rigorous research program. We seek applicants pursuing research directions with significant conceptual and/or empirical integration across traditional disciplinary boundaries. The appointee could be either an experimentalist or theorist. The successful candidate will join the Neuroscience Institute and may also join a department appropriate to the individual's background and interests, with possibilities including (but not limited to) Psychology, Molecular Biology, Mathematics, Physics, Electrical Engineering and Computer Science. Applicants should be prepared to teach courses both at the undergraduate and graduate levels in neuroscience.

Please submit a curriculum vitae, a brief research description, and contact information for three references at <http://jobs.princeton.edu>, requisition #1300694. Applications will be considered on a rolling basis, and the search will remain open until the position is filled.

Tenure Track Assistant Professor Position

The Princeton Neuroscience Institute invites applications for a tenure track appointment at the assistant professor level to begin on or after September 2014.

Key selection criteria will be research excellence, originality of science, and future impact on the field of neuroscience and related disciplines. We seek applicants pursuing research directions with significant conceptual and/or empirical integration across traditional disciplinary boundaries. The successful candidate will join the Neuroscience Institute and may also join a department appropriate to the individual's background and interests, with possibilities including (but not limited to) Psychology, Molecular Biology, Mathematics, Physics, Electrical Engineering and Computer Science. Applicants should be prepared to teach courses both at the undergraduate and graduate levels in neuroscience.

Please submit a curriculum vitae, a brief research description, and contact information for three references at <http://jobs.princeton.edu>, requisition #1300702. Applications will be considered on a rolling basis, and the search will remain open until the position is filled.

Position in Theoretical Neuroscience

The Princeton Neuroscience Institute invites applications for a tenure track appointment at the assistant professor level to begin on or after September 2014.

Key selection criteria will be research excellence, originality of science, and future impact on the field of neuroscience and related disciplines. The appointee would be expected to make contributions to theoretical and/or computational neuroscience and to engage with theoretically, computationally, and empirically inclined neuroscience researchers and trainees across a wide range of departments. The successful candidate will join the Neuroscience Institute and may also join a department appropriate to the individual's background and interests, with possibilities including (but not limited to) Psychology, Molecular Biology, Mathematics, Physics, Electrical Engineering and Computer Science. The applicant would be expected to participate in the graduate and undergraduate training programs in neuroscience and should be prepared to teach courses as part of each.

Please submit a curriculum vitae, a brief research description, and contact information for three references at <http://jobs.princeton.edu>, requisition #1300701. Applications will be considered on a rolling basis, and the search will remain open until the position is filled.

POSITIONS OPEN



ASSISTANT PROFESSOR Florida State University

Department of Chemistry and Biochemistry

The Department of Chemistry and Biochemistry at Florida State University seeks to fill a tenure-track faculty position at the Assistant Professor level beginning August 5, 2014. The Department is particularly interested in individuals with research interests in organic synthesis and/or chemical biology. Appointees will be expected to develop a vigorous, externally supported research program and to teach both undergraduate and graduate courses. Successful candidates will have a Ph.D. and postdoctoral training in a relevant field. Application materials, including a cover letter, curriculum vitae, research proposal, statement of teaching philosophy, and the names of at least three references, should be submitted online at **website: <http://facsearch.chem.fsu.edu>**. Review of applications will begin on November 1, 2013, and continue until the position is filled. *Florida State University is an Equal Opportunity Employer; minority candidates and women are especially encouraged to apply.*

ASSOCIATE/FULL PROFESSOR in Pharmaceutics

The Department of Pharmaceutical Sciences in the College of Pharmacy at the University of Tennessee Health Science Center in Memphis, TN, is seeking an outstanding applicant for a 12-month full-time, tenure-track faculty position at the associate or full professor level that is state supported. The successful candidate is expected to have a well-funded strong research program in the area of pharmaceutics with specialization in physical pharmacy, drug delivery, gene therapy, nanotechnology, nanomedicine, regenerative medicine, bioimaging, biosensors, or other related disciplines. The successful candidate is expected to have a Ph.D. or equivalent degree, the ability to maintain competitive extramural funding, a commitment to excellence in teaching pharmaceutics in Pharm.D. and graduate programs, and have excellent oral and written communication skills. Applications will be accepted until the position is filled.

Please submit a cover letter, curriculum vitae, summary of research and teaching interests, names and contact information of three references to: **Dr. John Buolamwini, Chair of Faculty Search Committee, Department of Pharmaceutical Sciences, 847 Monroe Avenue, Suite 327, Memphis, TN 38163, via e-mail: jbuolamw@uthsc.edu**.

The University of Tennessee Health Science Center is located in Memphis, TN, an economically vibrant center, with a metropolitan population of more than 1.3 million, reflecting the richness along the bluffs of the mighty Mississippi River. The College of Pharmacy is located in a new, six-story 187,000 square-foot building with state-of-the-art research space and teaching facility on the Health Science Center complex.

The University of Tennessee Health Science Center is an Equal Opportunity/Affirmative Action Employer.

FISHERIES BIOLOGIST and a MARINE BIOLOGIST

Georgia Southern University's Department of Biology invites applications for two positions: a Fisheries Biologist and a Marine Biologist. The positions require teaching, service, and research responsibilities as well as a terminal degree. The full text advertisements, including information about the department, faculty, and the complete position announcement with all qualifications and application instructions, is available at **website: <http://www.bio.georgiasouthern.edu>**. Deadline for applications is November 8, 2013. The preferred position starting date is August 1, 2014. *Georgia is an open records state. Georgia Southern is an Affirmative Action/Equal Opportunity Institution. Individuals who need reasonable accommodations under the ADA to participate in the search process should contact the Associate Provost.*

POSITIONS OPEN

TENURE-TRACK ASSISTANT PROFESSORSHIP University of Minnesota – Twin Cities

The Department of Biochemistry, Molecular Biology and Biophysics invites applications for a full-time, tenure-track Assistant Professor position to begin on or around July 1, 2014. We are particularly interested in candidates with expertise in transcriptional or post-transcriptional gene regulation, although all types biomedical research will be considered. The successful candidate will be expected to develop a strong, externally funded research program and contribute to the interdisciplinary undergraduate, graduate, and professional teaching programs. Cross-disciplinary collaboration is also an asset. All candidates must have a Ph.D. and/or M.D. degree, applicable postdoctoral experience, and a strong publication record. The successful candidate will receive a start-up package and a salary commensurate with education and experience.

Reviews will begin immediately and continue until the position is filled. Please apply online at **website: <http://employment.umn.edu>**, click on "Search Postings", and enter 187313 into the requisition number field. Please attach a full curriculum vitae, a two-page research overview, and a one-page teaching statement. Three letters of recommendation that consider both research and teaching potential should be sent directly from the referee to: **the Faculty Search Committee, c/o Ms. Ann Johnson, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 6-155 Jackson Hall, 321 Church Street S.E., Minneapolis, MN 55455** or as an attachment to e-mail: **swans143@umn.edu**.

The University of Minnesota provides equal access to and opportunity in its programs, facilities, and employment without regard to race, color, creed, religion, national origin, gender, age, marital status, disability, public assistance status, veteran status, sexual orientation, gender identity, or gender expression. The University supports the work-life balance of its faculty and especially encourages applications from women and members of underrepresented groups.

ASSISTANT PROFESSOR-ALL AREAS Princeton University Department of Chemistry

The Department of Chemistry at Princeton University invites applications for a tenure-track assistant professor position in all areas of chemistry. We seek faculty members who will create a climate that embraces excellence and diversity, with a strong commitment to teaching and mentoring that will enhance the work of the department and attract and retain students of all races, nationalities, and genders. We strongly encourage applications from members of all underrepresented groups. Candidates are expected to have completed the Ph.D. in chemistry or a related field at the time of appointment. Applicants should submit a description of research interests, curriculum vitae, a list of publications, and contact information for three references online at **website: <http://jobs.princeton.edu/applicants/Central?quickFind=64207>**. The search committee will begin review of applications on October 17, 2013 and will continue until the position is filled.

Princeton University is an Equal Opportunity Employer and complies with applicable EEO and Affirmative Action regulations.

ASSISTANT PROFESSOR OF BIOLOGY Saint Anselm College Manchester, NH

The Department of Biology invites applications for a tenure-track Assistant Professor position beginning August 2014. A Ph.D. and support of the College's mission are required. The successful candidate will teach Comparative Anatomy (with lab), Human Anatomy and Physiology (with lab), and a third course in their specific area of expertise. Continued research activities and mentoring of undergraduates are also integral to this position. Qualified individuals should submit a cover letter and curriculum vitae online at **website: <http://www.anselm.edu/hr>**. Three letters of recommendation should be submitted to **Dr. Donald Rhodes, e-mail: drhodes@anselm.edu**, no later than December 1, 2013.

Successful candidates will be able to assist the college to further its strategic goals for institution-wide diversity and inclusiveness.

POSITIONS OPEN

TENURE-TRACK ASSISTANT/ASSOCIATE PROFESSOR

The Department of Physics at the University of Miami invites applications from highly qualified persons for a tenure-track position in Condensed Matter Physics at the Assistant or Associate Professor rank to begin fall 2014. Experimental candidates are particularly encouraged to apply. Current departmental research in this area involves electronic, magnetic, thermal, and superconductive properties of novel materials with potential device applications. Candidates must have a Ph.D. in physics or related field, a demonstrated record of research achievements, and a strong commitment to teaching and mentoring students at the undergraduate and graduate levels. The physics department is located within the University's attractive Coral Gables campus in the greater Miami area, and has a wide-ranging research expertise and established Ph.D. program. Application materials, including curriculum vitae with list of publications and statement of research plans, should be sent electronically (as a single PDF) to e-mail: **barnes@physics.miami.edu** or to: **Professor Stewart E. Barnes, CM Search Committee Chair, Department of Physics, University of Miami, Knight Physics Building, Coral Gables, FL 33124**. Applicants should arrange for three letters of recommendation to be sent to the same address. Review of applications will begin on November 18, 2013 and continue until the position is filled. *The University of Miami is an Equal Opportunity/Affirmative Action Employer that values diversity and has progressive work-life policies. Women, persons with disabilities, and members of other underrepresented groups are encouraged to apply.*

TENURED OR TENURE-TRACK FACULTY POSITION IN FUNCTIONAL GENOMICS

The Department of Biology at The Johns Hopkins University is seeking to hire talented new faculty who apply genomic and bioinformatic approaches to investigate biological problems in creative and innovative ways. The ideal candidate will have an established record of funding, a vibrant research program and enthusiasm for collaboration as well as undergraduate and graduate teaching. Appointments at the Associate Professor level are preferred, though higher and lower ranks will be considered on a case-by-case basis. This hire is part of a major, multi-year expansion of the Department, including the addition of new faculty, infrastructure, and research space. New faculty will be able to forge strong connections with scientists throughout the Johns Hopkins community and influence the future direction of biomedical research at Hopkins. More information about the Department can be found at **website: <http://www.bio.jhu.edu>**.

Please submit your application files including a curriculum vitae, statement of current and future research, and statement of teaching interests and philosophy, and arrange to have three confidential letters of recommendation submitted through **website: <http://www.apply.interfolio.com/23024>**.

Review of submitted applications will begin November 1, 2013 and will continue until the position is filled. *Johns Hopkins University is an Equal Opportunity/Affirmative Action Employer and actively encourages interest from minorities and women.*

DEPARTMENT HEAD Fish and Wildlife Sciences. University of Idaho - Moscow, ID

The College of Natural Resources at the University of Idaho seeks an exceptional individual to serve as Head of the Department of Fish and Wildlife Sciences. The successful applicant will serve as leader of an active, collegial department, work with faculty and staff to foster new research initiatives, mentor faculty, and help our students to succeed. The incumbent must possess a doctorate in wildlife, fisheries, or a natural resource discipline (or closely related field) with an outstanding record of research, teaching, and outreach sufficient to merit a national and international reputation appropriate for the rank of **PROFESSOR**. For a complete description and to apply online, please visit **website: <http://apptkr.com/389675>** by October 25, 2013. *Equal Opportunity Employer.*